**LCMS-8040**

**Summary**
Evaluation and quantitation of a variety of cannabinoids on an LCMS-8040 triple quadrupole mass spectrometer.

**Method**
Cannabichromene (CBC), cannabigerol (CBG) and Δ9-THC Acid A (THCA) standards were purchased from Restek (Bellefonte, PA). Cannabinol (CBN), Δ8-THC (d8-THC), Δ9-THC (d9-THC), Δ9-THC-D3 (d9-THC-D3) and Cannabidiol (CBD) were purchased from Cerilliant (Round Rock, TX). A stock concentration of 1 µg/mL for each standard was used for MRM optimization. The standards were combined into one solution and then serially diluted with 70/30 (%) methanol/water using 2-fold dilutions yielding concentrations ranging from 8192 ng/mL to 1 ng/mL. The IS (d9-THC-D3) was added to all levels. The standards (Figure 1) were transferred to autosampler vials and injected into a Nexera-LCMS-8040 system for analysis.

A Thermo Hypersil Gold (1.9 µm x 2.1 mm x 100 mm) was used with a binary gradient of 5 mM ammonium acetate in water and acetonitrile. The gradient conditions are shown in Figure 2. The flow rate was 0.5 mL/min with a run time of 6.8 min. The column temperature was 45°C and the injection volume was 5 µL.

**LCMS-8040 Analysis**
Dual ion electrospray ionization (DUIS) in both positive and negative modes was used for ionization of the analytes on the LCMS-8040. Multiple reaction monitoring (MRM) in positive and negative modes was used for analysis. At least one reference ion was monitored for each analyte. Details of the MS parameters are shown in Table 1.

---

**Figure 1.** Chemical structures for cannabinoids.
Summary
Evaluation and quantitation of a variety of cannabinoids on an LCMS-8040 triple quadru-pole mass spectrometer.

Method
Cannabichromene (CBC), cannabigerol (CBG) and ∆9-THC Acid A (THCA) standards were purchased from Restek (Bellefonte, PA). Cannabinol (CBN), ∆8-THC (d8-THC), ∆9-THC (d9-THC), ∆9-THC-D3 (d9-THC-D3) and Cannabidiol (CBD) were purchased from Cerilliant (Round Rock, TX). A stock concentration of 1 µg/mL for each standard was used for MRM optimization. The standards were combined into one solution and then serially diluted with 70/30 (%/%) methanol/water using 2-fold dilutions yielding concentrations ranging from 8192 ng/mL to 1 ng/mL. The IS (d9-THC-D3) was added to all levels. The standards (Figure 1) were transferred to autosampler vials and injected into a Nexera-LCMS-8040 system for analysis.

A Thermo Hypersil Gold (1.9 µm x 2.1 mm x 100 mm) was used with a binary gradient of 5 mM ammonium acetate in water and acetonitrile. The gradient conditions are shown in Figure 2. The flow rate was 0.5 mL/min with a run time of 6.8 min. The column temperature was 45 °C and the injection volume was 5 µL.

LCMS-8040 Analysis
Dual ion electrospray ionization (DUIS) in both positive and negative modes was used for ionization of the analytes on the LCMS-8040. Multiple reaction monitoring (MRM) in positive and negative modes was used for analysis. At least one reference ion was monitored for each analyte. Details of the MS parameters are shown in Table 1.

Results and Discussion
Peaks were smoothed using the standard method set to 2 counts and 2 sec for width. The auto integration setting was used with a setting of 1 max peak and 6 sec width. d8-THC was integrated by turning off peak integration from 4.9-5.3 minutes. Linearity was achieved from 1-8192 ng/mL for CBG, 2-4096 ng/mL for CBN, 4-4096 ng/mL for d9-THC, 8-8192 ng/mL for d8-THC, 64-4096 ng/mL for THCA, 4-8192 ng/mL for CBD and CBC. The calibration curve was weighted using 1/C and not forced through zero. No standards were excluded over the included range and r² values ranged from 0.998-0.999. A representative chromatogram and calibration curve for each analyte are shown in Figures 4-11.

Table 1. MS Interface parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interface</td>
<td>DUIS</td>
</tr>
<tr>
<td>Nebulizing gas flow</td>
<td>2 L/min</td>
</tr>
<tr>
<td>Drying gas flow</td>
<td>15 L/min</td>
</tr>
<tr>
<td>DL Temperature</td>
<td>300 °C</td>
</tr>
<tr>
<td>Heat Block Temperature</td>
<td>500 °C</td>
</tr>
<tr>
<td>Polarity</td>
<td>(+) CBD, CBG, CBN, d8-THC, d9-THC, CBC (-) THCA</td>
</tr>
</tbody>
</table>

Figure 2. Gradient conditions.

Figure 3. Representative chromatogram cannabinoids at 64 ng/mL.

Figure 4. Chromatogram and calibration curve for cannabidiol (CBD).
Evaluation and quantitation of a variety of cannabinoids on an LCMS-8040 triple quadrupole mass spectrometer.

**Method**

Cannabichromene (CBC), cannabigerol (CBG) and ∆9-THC Acid A (THCA) standards were purchased from Restek (Bellefonte, PA). Cannabinol (CBN), ∆8-THC (d8-THC), ∆9-THC (d9-THC), ∆9-THC-D3 (d9-THC-D3) and Cannabidiol (CBD) were purchased from Cerilliant (Round Rock, TX). A stock concentration of 1 µg/mL for each standard was used for MRM optimization. The standards were combined into one solution and then serially diluted with 70/30 (% methanol/water using 2-fold dilutions yielding concentrations ranging from 8192 ng/mL to 1 ng/mL. The IS (d9-THC-D3) was added to all levels. The standards were transferred to autosampler vials and injected into a Nexera-LCMS-8040 system for analysis.

A Thermo Hypersil Gold (1.9 µm x 2.1 mm x 100 mm) was used with a binary gradient of 5 mM ammonium acetate in water and acetonitrile. The gradient conditions are shown in Figure 2. The flow rate was 0.5 mL/min with a run time of 6.8 min. The column temperature was 45 ºC and the injection volume was 5 µL.

**LCMS-8040 Analysis**

Dual ion electrospray ionization (DUIS) in both positive and negative modes was used for ionization of the analytes on the LCMS-8040. Multiple reaction monitoring (MRM) in positive and negative modes was used for analysis. At least one reference ion was monitored for each analyte. Details of the MS parameters are shown in Table 1.

**Results and Discussion**

Peaks were smoothed using the standard method set to 2 counts and 2 sec for width. The auto integration setting was used with a setting of 1 max peak and 6 sec width. d8-THC was integrated by turning off peak integration from 4.9-5.3 minutes. Linearity was achieved from 1-8192 ng/mL for CBG, 2-4096 ng/mL for CBN, 4-4096 ng/mL for d9-THC, 8-8192 ng/mL for d8-THC, 64-4096 ng/mL for THCA, 4-8192 ng/mL for CBD and CBC. The calibration curve was weighted using 1/C and not forced through zero. No standards were excluded over the included range and r² values ranged from 0.998-0.999. A representative chromatogram and calibration curve for each analyte are shown in Figures 4-11.

**Figure 5.** Chromatogram and calibration curve for cannabinol (CBN).

**Figure 6.** Chromatogram and calibration curve for d8-THC.

**Figure 7.** Chromatogram and calibration curve for d9-THC.
Evaluation and quantitation of a variety of cannabinoids on an LCMS-8040 triple quadropole mass spectrometer.

Method
Cannabichromene (CBC), cannabigerol (CBG) and ∆9-THC Acid A (THCA) standards were purchased from Restek (Bellefonte, PA). Cannabinol (CBN), ∆8-THC (d8-THC), ∆9-THC (d9-THC), ∆9-THC-D3 (d9-THC-D3) and Cannabidiol (CBD) were purchased from Cerilliant (Round Rock, TX). A stock concentration of 1 µg/mL for each standard was used for MRM optimization. The standards were combined into one solution and then serially diluted with 70/30 (%)/methanol/water using 2-fold dilutions yielding concentrations ranging from 8192 ng/mL to 1 ng/mL. The IS (d9-THC-D3) was added to all levels. The standards (Figure 1) were transferred to autosampler vials and injected into a Nexera-LCMS-8040 system for analysis.

A Thermo Hypersil Gold (1.9 µm x 2.1 mm x 100 mm) was used with a binary gradient of 5 mM ammonium acetate in water and acetonitrile. The gradient conditions are shown in Figure 2.

The flow rate was 0.5 mL/min with a run time of 6.8 min. The column temperature was 45 ºC and the injection volume was 5 µL.

LCMS-8040 Analysis
Dual ion electrospray ionization (DUIS) in both positive and negative modes was used for ionization of the analytes on the LCMS-8040. Multiple reaction monitoring (MRM) in positive and negative modes was used for analysis. At least one reference ion was monitored for each analyte. Details of the MS parameters are shown in Table 1.

Results and Discussion
Peaks were smoothed using the standard method set to 2 counts and 2 sec for width. The auto integration setting was used with a setting of 1 max peak and 6 sec width. d8-THC was integrated by turning off peak integration from 4.9-5.3 minutes. Linearity was achieved from 1-8192 ng/mL for CBG, 2-4096 ng/mL for CBN, 4-4096 ng/mL for d9-THC, 8-8192 ng/mL for d8-THC, 64-4096 ng/mL for THCA, 4-8192 ng/mL for CBD and CBC. The calibration curve was weighted using 1/C and not forced through zero. No standards were excluded over the included range and r² values ranged from 0.998-0.999. A representative chromatogram and calibration curve for each analyte are shown in Figures 4-11.

Figure 8. Chromatogram and calibration curve for cannabigerol (CBG).

Figure 9. Chromatogram and calibration curve for cannabichromene (CBC).

Figure 10. Chromatogram and calibration curve for d9-THC Acid A (THCA).

Conclusion
A method for rapid quantitation of various cannabinoids utilizing a Nexera X2 UHPLC coupled to an LCMS-8040 triple quadrupole mass spectrometer was developed. An 8 minute method was optimized, with the main limiting factor being the separation of d8-THC and d9-THC due to their structure being nearly identical. It is important to note that these results were achieved using standards without matrix, but the ability of the Nexera X2 system and LCMS-8040 mass spectrometer to enable simultaneous, accurate measurement of many of the cannabinoids of interest is highlighted by this method.
Evaluation and quantitation of a variety of cannabinoids on an LCMS-8040 triple quadrupole mass spectrometer.

Method
Cannabichromene (CBC), cannabigerol (CBG) and ∆9-THC Acid A (THCA) standards were purchased from Restek (Bellefonte, PA). Cannabinol (CBN), ∆8-THC (d8-THC), ∆9-THC (d9-THC), ∆9-THC-D3 (d9-THC-D3) and Cannabidiol (CBD) were purchased from Cerilliant (Round Rock, TX). A stock concentration of 1 µg/mL for each standard was used for MRM optimization. The standards were combined into one solution and then serially diluted with 70/30 (%)/methanol/water using 2-fold dilutions yielding concentrations ranging from 8192 ng/mL to 1 ng/mL. The IS (d9-THC-D3) was added to all levels. The standards (Figure 1) were transferred to autosampler vials and injected into a Nexera-LCMS-8040 system for analysis.

A Thermo Hypersil Gold (1.9 µm x 2.1 mm x 100 mm) was used with a binary gradient of 5 mM ammonium acetate in water and acetonitrile. The gradient conditions are shown in Figure 2. The flow rate was 0.5 mL/min with a run time of 6.8 min. The column temperature was 45 ºC and the injection volume was 5 µL.

LCMS-8040 Analysis
Dual ion electrospray ionization (DUIS) in both positive and negative modes was used for ionization of the analytes on the LCMS-8040. Multiple reaction monitoring (MRM) in positive and negative modes was used for analysis. At least one reference ion was monitored for each analyte. Details of the MS parameters are shown in Table 1.

Results and Discussion
Peaks were smoothed using the standard method set to 2 counts and 2 sec for width. The auto integration setting was used with a setting of 1 max peak and 6 sec width. d8-THC was integrated by turning off peak integration from 4.9-5.3 minutes. Linearity was achieved from 1-8192 ng/mL for CBG, 2-4096 ng/mL for CBN, 4-4096 ng/mL for d9-THC, 8-8192 ng/mL for d8-THC, 64-4096 ng/mL for THCA, 4-8192 ng/mL for CBD and CBC. The calibration curve was weighted using 1/C and not forced through zero. No standards were excluded over the included range and r² values ranged from 0.998-0.999. A representative chromatogram and calibration curve for each analyte are shown in Figures 4-11.

Conclusion
A method for rapid quantitation of various cannabinoids utilizing a Nexera X2 UHPLC coupled to an LCMS-8040 triple quadrupole mass spectrometer was developed. An 8 minute method was optimized, with the main limiting factor being the separation of d8-THC and d9-THC due to their structure being nearly identical.

It is important to note that these results were achieved using standards without matrix, but the ability of the Nexera X2 system and LCMS-8040 mass spectrometer to enable simultaneous, accurate measurement of many of the cannabinoids of interest is highlighted by this method.
Results and Discussion

Method

A method for rapid quantitation of various cannabinoids on an LCMS-8040 triple quadrupole mass spectrometer was developed. An 8 pole mass spectrometer was selected for its the ability of the Nexera X2 system and LCMS-8040 to enable simultaneous, accurate measurement of many of the cannabinoids on interest is highlighted by this method.

Evaluation and quantitation of a variety of cannabinoids on an LCMS-8040 triple quadrupole mass spectrometer was achieved using standards without matrix, but limiting factor being the separation of d8-THC due to their structure being nearly identical.

Method

The columns and conditions of a LCMS-IT-TOF was used with a binary gradient of 5 mM ammonium acetate in water and acetonitrile.

The auto integration setting was used with a method set to 2 counts and 2 sec for width. Peaks were smoothed using the standard setting of 1 max peak and 6 sec width. d8-THC was integrated by turning off peak integration and then serially diluted with 70/30 (%)

The column temperature was 45 º C.

The flow rate was 0.5 mL/min with a run time of 6.8 min. The standards were combined into one solution and the injection volume was 5 µL.

Peaks were smoothed using the standard setting of 1 max peak and 6 sec width. d8-THC was integrated by turning off peak integration and then serially diluted with 70/30 (%)

Table 1

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Retention Time (min)</th>
<th>Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d9-THC-D3</td>
<td>4.9-5.3</td>
<td>1-8192</td>
</tr>
<tr>
<td>CBD</td>
<td>16.5-18.5</td>
<td>1-8192</td>
</tr>
<tr>
<td>CBG</td>
<td>22.0-23.0</td>
<td>4-8192</td>
</tr>
<tr>
<td>∆8-THC (d8-THC)</td>
<td>23.0-24.0</td>
<td>4-8192</td>
</tr>
<tr>
<td>∆9-THC (d9-THC)</td>
<td>25.0-26.5</td>
<td>4-8192</td>
</tr>
<tr>
<td>THCA</td>
<td>27.5-28.5</td>
<td>4-8192</td>
</tr>
<tr>
<td>∆9-THC Acid A</td>
<td>29.0-30.0</td>
<td>4-8192</td>
</tr>
<tr>
<td>CBC</td>
<td>31.0-32.0</td>
<td>4-8192</td>
</tr>
<tr>
<td>∆9-THC-D3</td>
<td>33.5-34.5</td>
<td>4-8192</td>
</tr>
<tr>
<td>CBN</td>
<td>36.0-37.0</td>
<td>4-8192</td>
</tr>
</tbody>
</table>
| Restek (Bellefonte, PA). A stock concentration of 1 µg/mL for ∆9-THC-D3 (d9-THC-D3) and Cannabidiol (CBD) were purchased from Cerilliant (Round Rock, TX).

The flow rate was 0.5 mL/min with a run time of 6.8 min. The standards were combined into one solution and the injection volume was 5 µL.