Introduction
Currently, HPLC with UV and photodiode array detection is widely applied for the analysis of cannabinoids in cannabis and hemp as the approach is relatively easy and inexpensive. With growing interest in cannabis- and hemp-based products and their inherent complexity, the analytical benefits of LC-MS, such as higher sensitivity, selectivity, and mass identification are increasingly recognized for the determination of cannabinoids.

Triple quadrupole LC-MS/MS methods have been applied to cannabis applications. When using multi-stage MS, a precursor ion is selected and fragmented in a collision cell. The collision-induced dissociation (CID) used in MS/MS can be used for structural determination. Fragmentation is also possible using single quadrupole MS, called source-induced dissociation (SID). SID is also referred to as in-source fragmentation or in-source CID (IS-CID). The information resulting from SID of cannabinoids can provide mass identification and specificity compared to HPLC/UV detectors.

For this study, an IS-CID method was developed using a single quadrupole MS with an integrated LC front end for chromatographic separation for 16 cannabinoids, including Δ9-tetrahydrocannabinol (Δ9-THC), Δ8-tetrahydrocannabinol (Δ8-THC), cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN), cannabicyclol (CBL), and their respective acidic forms etc. Both positive and negative scans and selected ion monitoring (SIM) were used simultaneously in the method and a MS library for the 16 cannabinoids was created for the rapid identification of target analytes in unknown samples.

Materials
A mixture of 16 cannabinoids was prepared using an 11-component mixture (Shimadzu 220-91239-21) with five additional individual cannabinoids obtained from Sigma Aldrich (Cerilliant). LCMS grade solvents, formic acid and ammonium formate were obtained from Honeywell. Degassed deionized water was used (>18.0 MΩ-cm). Standards were prepared by diluting with 90% mobile phase B and 10% DI.

HPLC-MS Conditions
The method conditions for both HPLC and MS are shown in Table 1.

Table 1: Method Conditions

<table>
<thead>
<tr>
<th>LC (2040C 3D)</th>
<th>MS (LCMS-2020)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column</strong></td>
<td>Shimadzu NexLeaf CBX II, 1.8 μm, 3.0 x 100mm (220-91525-75) Shimadzu NexLeaf CBX II Guard, 1.8 μm (220-91525-76)</td>
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<tr>
<td><strong>Mobile phase A</strong></td>
<td>0.1% formic acid and 5 mM ammonium formate in 100% water</td>
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<tr>
<td><strong>Mobile phase B</strong></td>
<td>0.1% formic acid in 50% methanol/50% acetonitrile</td>
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<tr>
<td><strong>Flow rate</strong></td>
<td>0.5 mL/min</td>
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<tr>
<td><strong>Oven</strong></td>
<td>30 °C</td>
</tr>
<tr>
<td><strong>Injection volume</strong></td>
<td>5 μL</td>
</tr>
<tr>
<td><strong>Gradient</strong></td>
<td>83% mobile phase B to 98% mobile phase B in 6.5 min.</td>
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Experimental Equipment
Experiments were performed using a Shimadzu integrated HPLC (2040C 3D) and a single quadrupole mass spectrometer detector (LCMS-2020) with electrospray ionization (ESI) interface.
Results and Discussion

Figure 1 shows the separation of 16 cannabinoids. Under the conditions of the experiment, neutral cannabinoids such as Δ9-THC, CBD, and CBL ionize in positive mode while their respective acidic forms ionize in negative mode. Although CBD and CBG co-elute from the column, their molecular weights differ and mass identification can be obtained from mass spectra as shown in Figure 2. In addition, Figure 3 shows that the SID fragmentation patterns from CBD and CBG are quite different using the method developed here. These more specific results show the advantage of LCMS over LC/UV in cannabinoids analysis.

![Figure 1: Separation chromatogram of 16 cannabinoid standards mixture (10 ppm each)](image1)

![Figure 2: Electrospray mass spectrum of CBD and CBG](image2)

Method Reproducibility

In-source fragmentation in single-quad MS is performed by manipulating the DC voltage of the Q-array, which is the first focusing optic after the desolvation line. It was noticed that increasing the Q-array voltage will produce more fragmentation, but continued voltage increases eventually result in an absence of fragmentation. In order to get the most reliable SID fragmentation for all 16 compounds in the study, Q-array DC voltage was optimized at different event times. Through trial and error, a Q-array DC voltage of 55 volts and an event time of 0.1 second were chosen for the method.

Experimental results show that even though some cannabinoids have the same mass and similar structure, different mass spectra patterns are obtained for the 16 compounds. In addition, SID fragmentation depends on sample matrix. Optimization of the fragmentation method may be necessary on a case-by-case basis.
To test the reproducibility and reliability of the method, four injections of the 16-cannabinoids mixture were performed. The mass spectrum of each cannabinoid was compared with mass spectra from the individual standards. Reproducible and consistent fragmentation was observed for the 16 cannabinoids from both individual and mixed standards.

Figures 4 and 5 are comparison results from Δ9-THC and CBL, respectively. Panel A in both figures is the spectrum from individual standard injections, and panel B shows the results from four injections of a mixed sample. As shown in both Fig. 4 and Fig. 5, very reproducible fragmentation is obtained from four injections of mixed 16 cannabinoids for both Δ9-THC and CBL, and fragmentation patterns are also consistent with spectra obtained from individual cannabinoid standard injections.

**Figure 4** Electrospray mass spectrum of Δ9-THC. Panel A is the result from single Δ9-THC standard injection; the left spectrum in panel A is the mass spectrum of precursor ions, the right spectrum in panel A is the mass spectrum of product ions from in-source fragmentation. Panel B is the comparison results of Δ9-THC from four injections of 16 mixed cannabinoids.
Figure 5 Electrospray mass spectrum of CBL. Panel A is the result from single CBL standard injection; the left spectrum in panel A is the mass spectrum of precursor ions, the right spectrum in panel A is the mass spectrum of product ions from in-source fragmentation. Panel B is the comparison results of CBL from four injections of 16 mixed cannabinoids.

Mass spectral library
A mass spectral library with both molecular weight and fragmentation information for 16 cannabinoids used in this study was created. Figure 6 is an example of a product ions spectrum of Δ9-THC built in the library. The spectral library can be a useful tool to identify unknown samples.
Conclusion
An in-source fragmentation method was developed using the Shimadzu single quadrupole MS to analyze 16 cannabinoids, including Δ9-tetrahydrocannabinol (Δ9-THC), Δ8-tetrahydrocannabinol (Δ8-THC), cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), cannabicyclol (CBL) and their respective acidic forms. This method shows the added benefit of mass spectrometry to the analysis of cannabinoids by providing more confident results with mass identification and library matching.