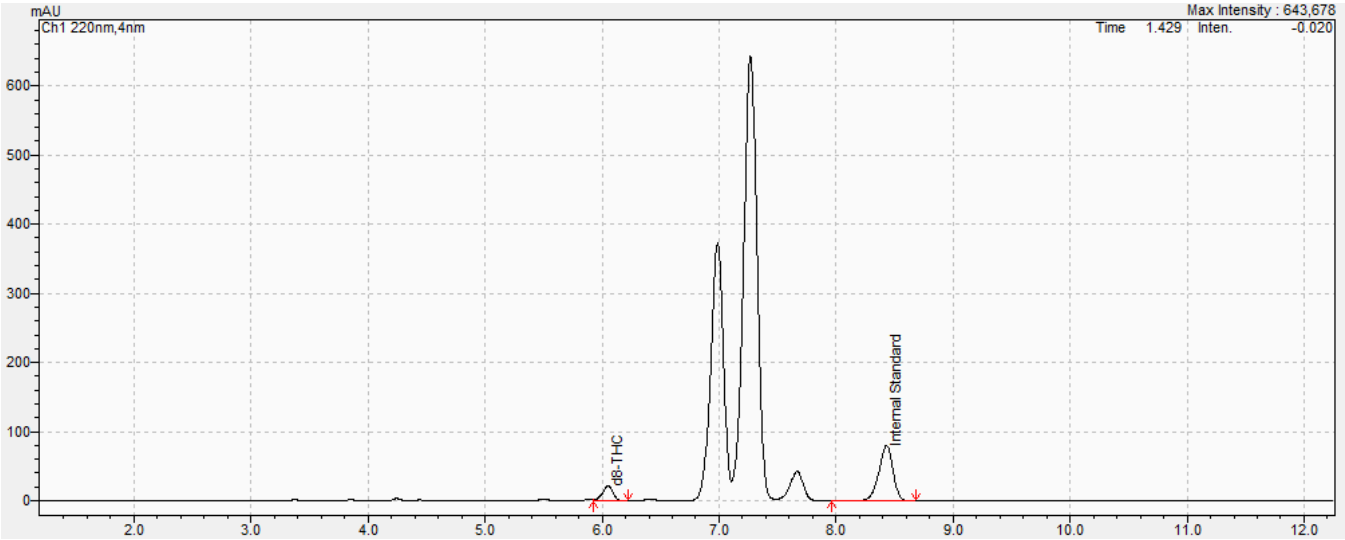
232 North Plaza Drive
Nicholasville, KY 40356

Colorado Chromatography Report 28May2021

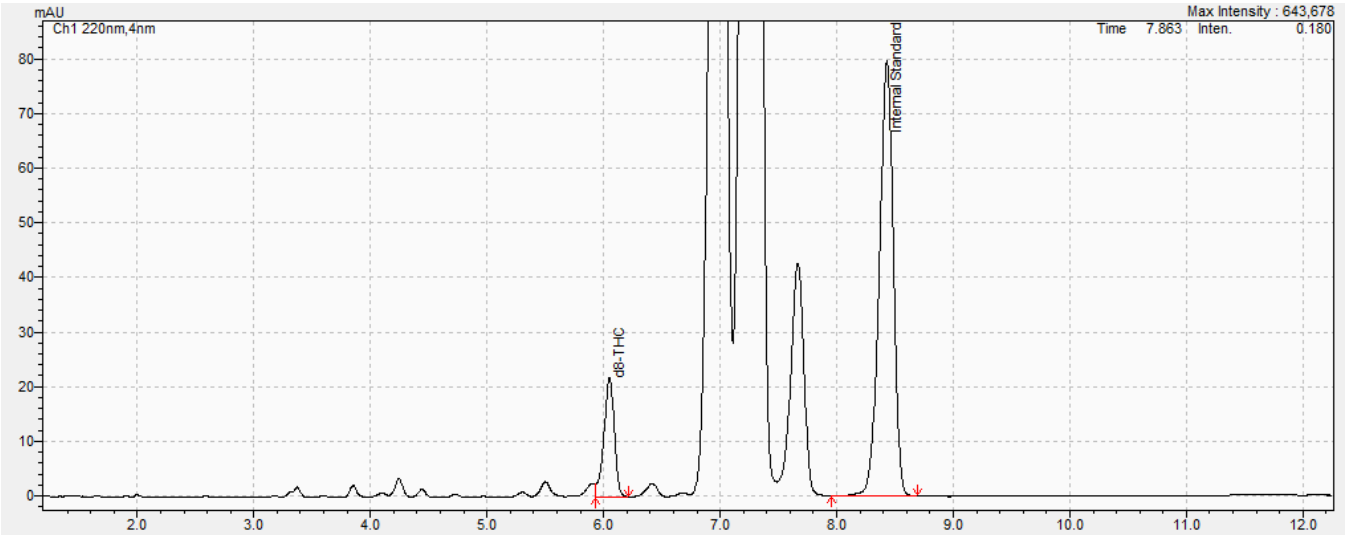
HPLC-PDA Data Analysis

Samples were initially analyzed by HPLC-PDA to identify and quantify cannabinoids in the mixture of 18 standard cannabinoids plus additional cannabinoids (*i.e.*, CBND, CBT, CBE, Δ^{10}-THC, $\Delta^{6a,10a}$-THC etc.). Cannabinoids were identified by comparison of their relative retention times (RRT) and the UV-spectral data. Samples were analyzed twice – once at a concentrated level to detect and quantify minor cannabinoids and once after dilution to detect and quantify major cannabinoids).

Sample ...2132



Sample ...2132 (Zoomed In)



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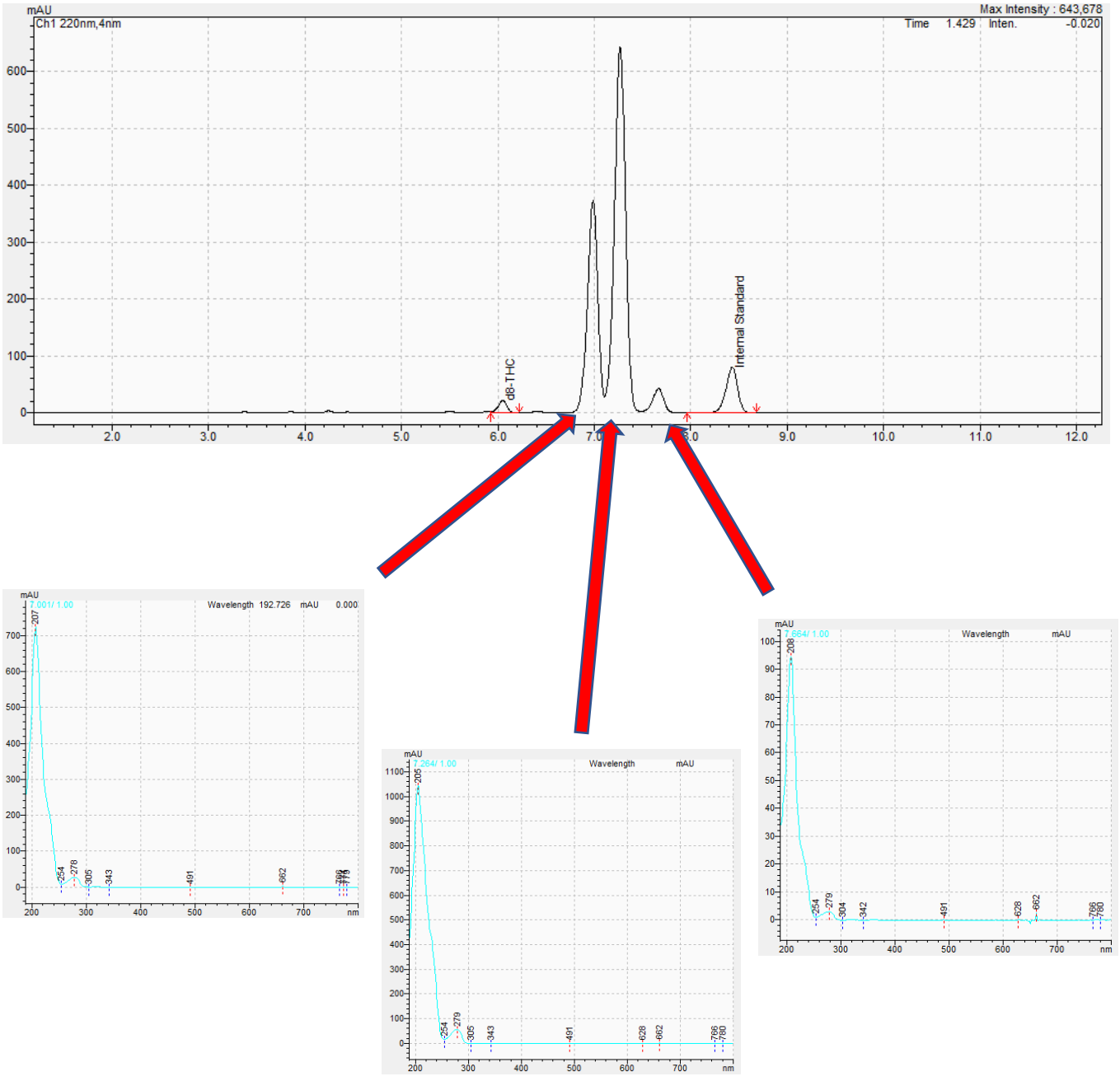
Δ^8-THC was the only cannabinoid identified from the reference standards analyzed by this method. The spectra of unknown peaks are displayed below. At least three unidentified substances eluted after Δ^8-THC indicating that they are more lipophilic than Δ^8-THC. If the molar absorptivities of these substances are similar to that of Δ^8-THC, then at least two of them are much more abundant than that of Δ^8-THC and the third at a retention time of about 7.7 min is about twice the abundance of Δ^8-THC.

Origins of PDA Spectral Data



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The PDA spectral data for these peaks appear similar to those of other cannabinoids such as Δ^8-THC, Δ^9-THC, and CBD without extended conjugation.

Since the major components of the sample were not identified by HPLC-PDA, we subjected the sample to LC-MS/MS analysis as shown on the following pages.

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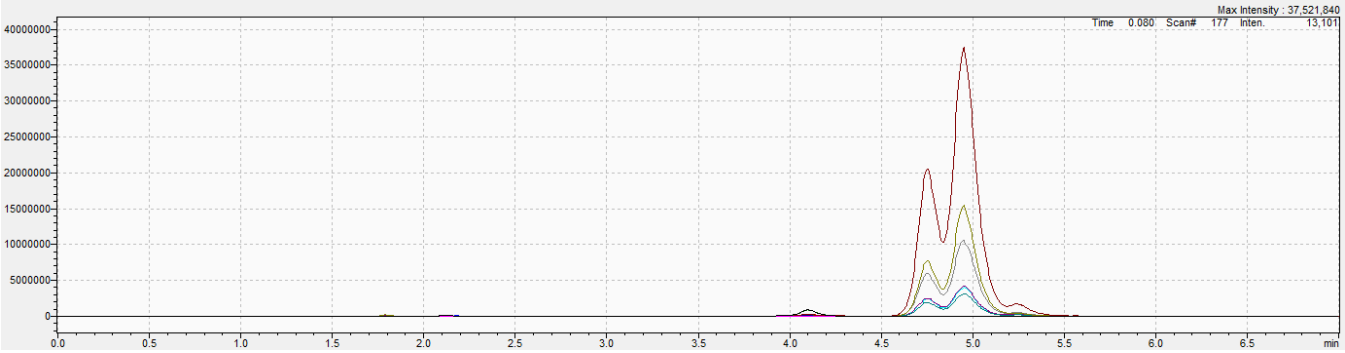
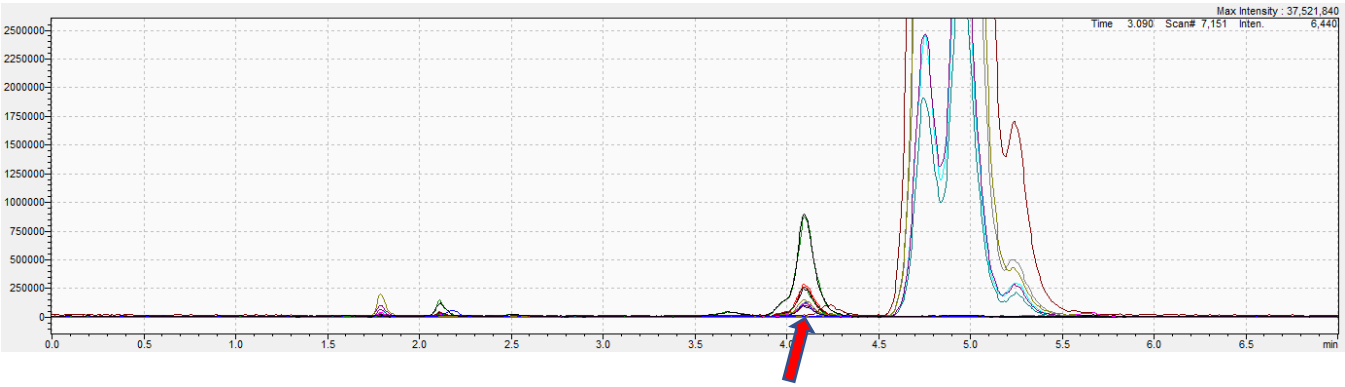
LC-MS/MS Data Analysis

Full Scan Data

Sample ...2132



Sample ...2132 (Zoomed In)



Δ8 THC

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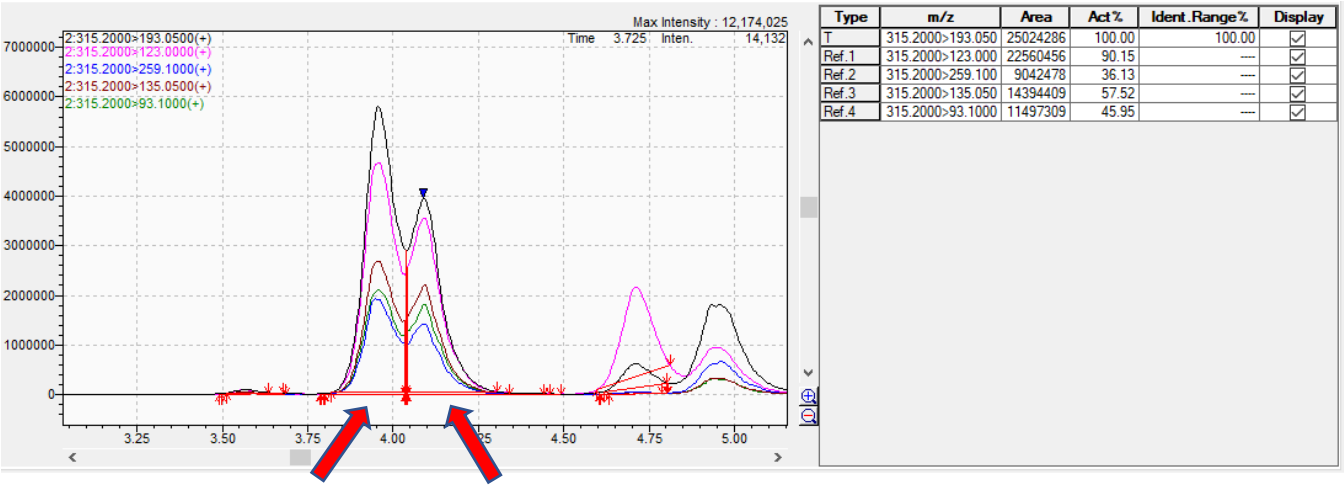
Data were then acquired in MRM (multiple reaction monitoring mode). The MRM transitions are listed to the right of the chromatogram. The “Act%” is the relative abundance (RA) of each MRM transition compared to the primary transition (calculated by area counts). To identify a compound, the relative abundances (RA) of the MRM transitions of the peak are compared to those of a reference standard.

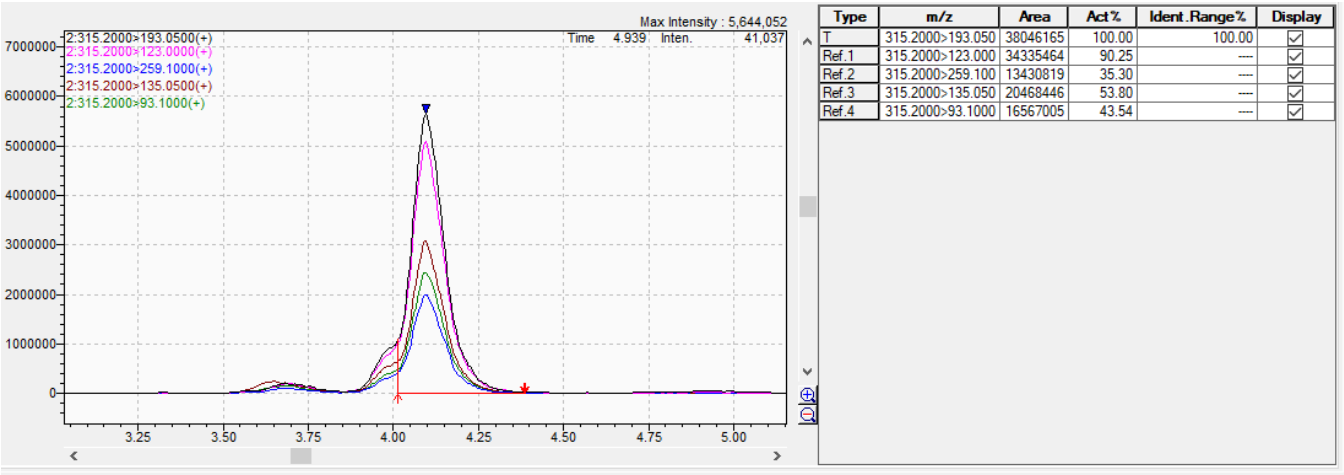
Calibration Mix containing cannabinoid standards.



Type	m/z	Area	Act%	Ident.Range%	Display
T	315.2000>193.050	25024286	100.00	100.00	☑
Ref.1	315.2000>123.000	22560456	90.15	---	☑
Ref.2	315.2000>259.100	9042478	36.13	---	☑
Ref.3	315.2000>135.050	14394409	57.52	---	☑
Ref.4	315.2000>93.1000	11497309	45.95	---	☑

Δ9 THC Δ8 THC

Sample ...2132



Type	m/z	Area	Act%	Ident.Range%	Display
T	315.2000>193.050	38046165	100.00	100.00	☑
Ref.1	315.2000>123.000	34335464	90.25	---	☑
Ref.2	315.2000>259.100	13430819	35.30	---	☑
Ref.3	315.2000>135.050	20468446	53.80	---	☑
Ref.4	315.2000>93.1000	16567005	43.54	---	☑

The presence of Δ^8-THC was confirmed by the ions and ion area ratios of the peak at 4.10 minutes in sample #2132. The peak just to the left of the peak for Δ^8-THC has ions characteristic of Δ^9-THC but we do not believe that identification criteria were met for this identification.

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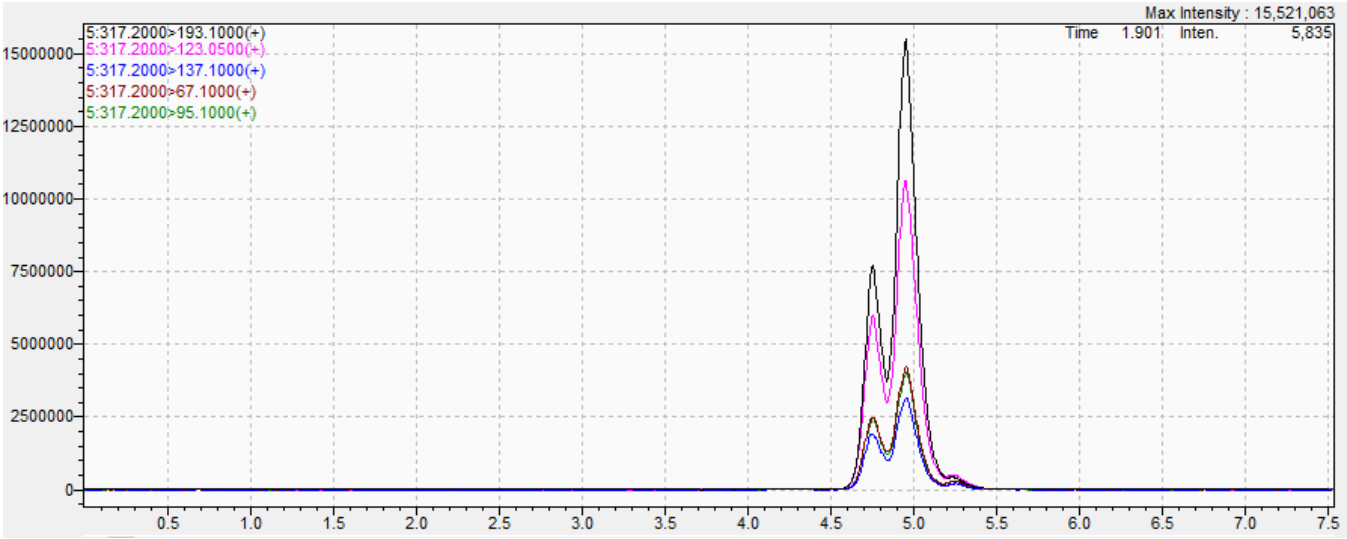
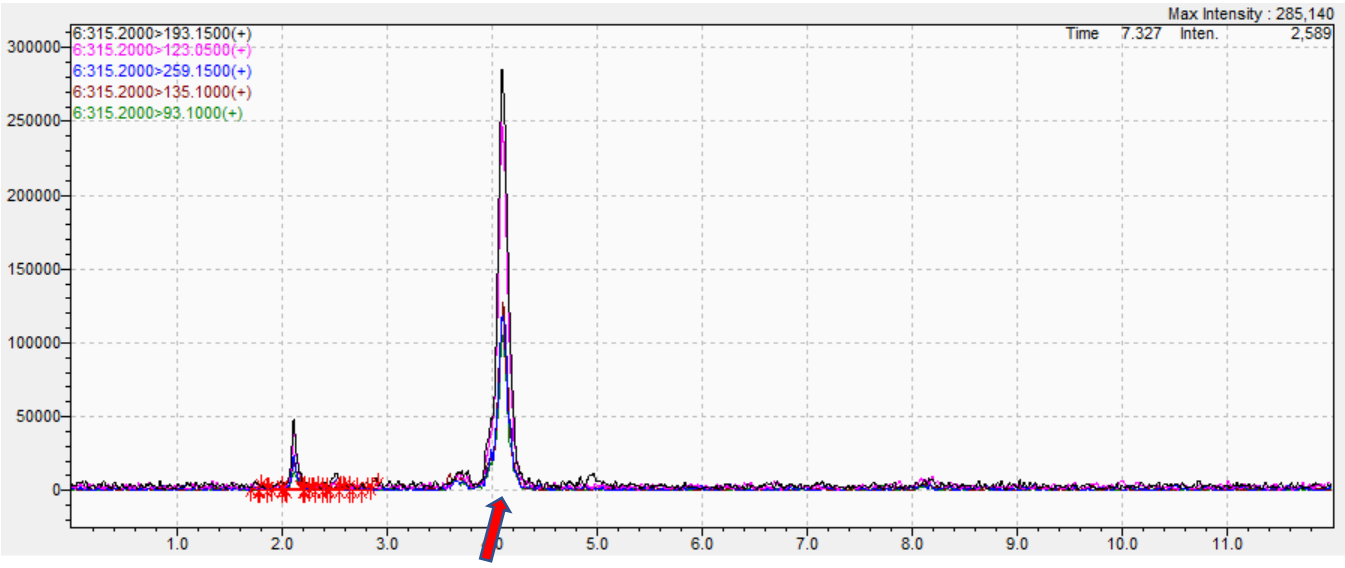
Ion chromatogram for *m/z* 317.2 in positive ionization mode



These peaks have masses and fragmentation patterns similar to those of CBG but the retention times are incorrect for CBG. The apparent molecular mass of the three substances eluting between 4.5 and 5.3 minutes is 316. This mass is identical to that of various isomers of hexahydrocannabinol.

Ion chromatogram for *m/z* 315.2 in positive ionization mode



Δ^8-THC

The identification of Δ^8-THC was confirmed.

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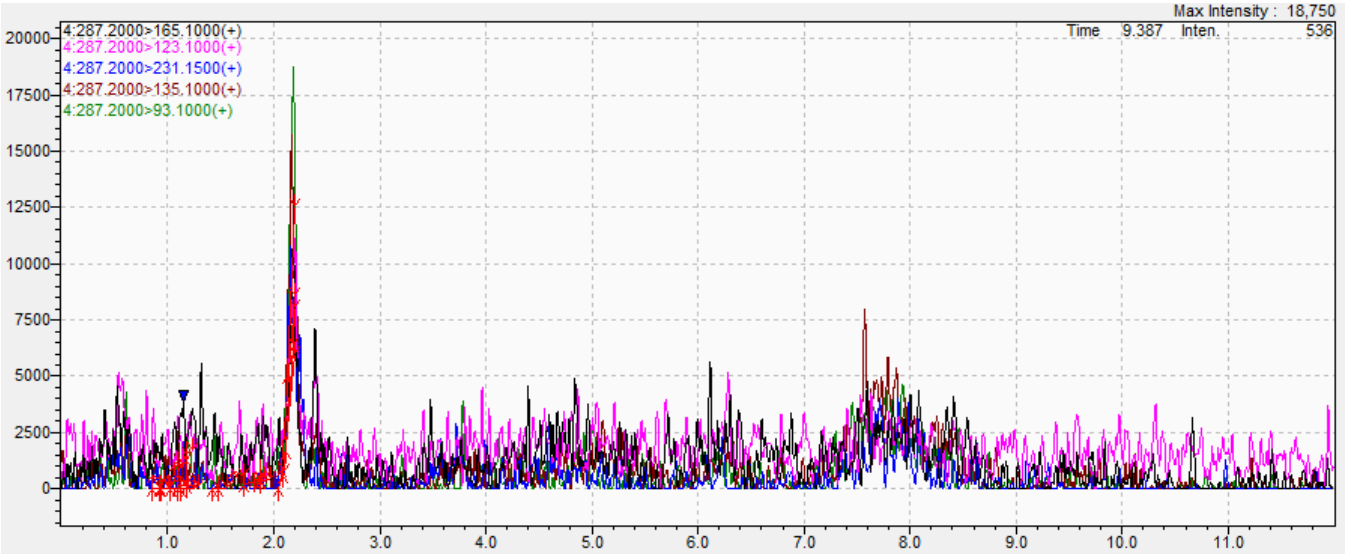
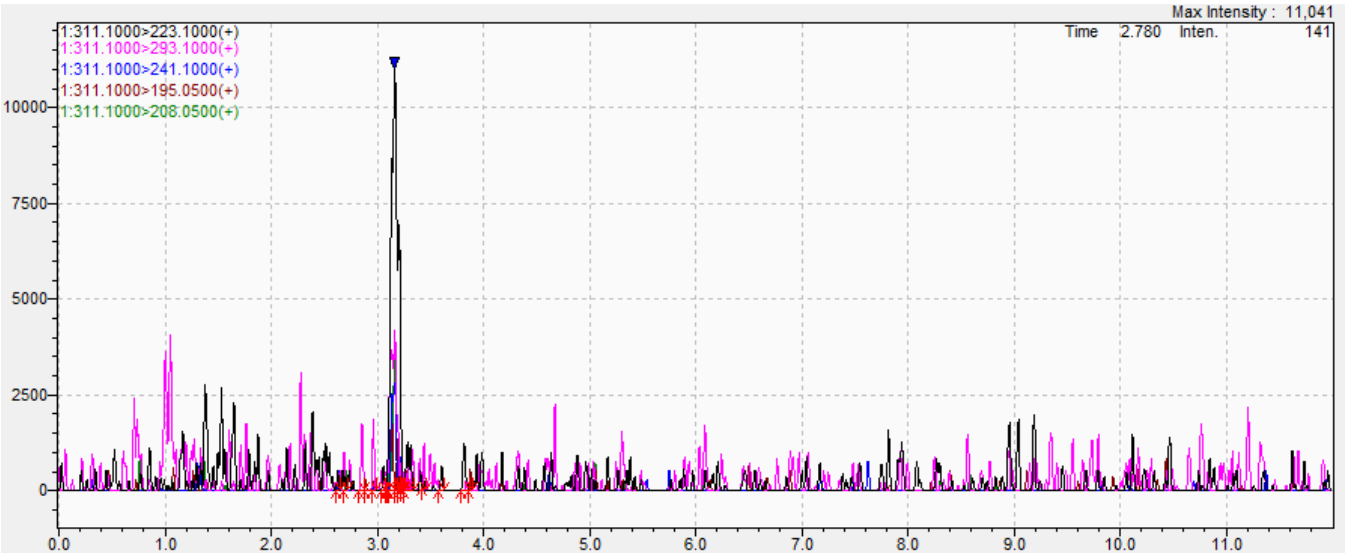
Ion chromatogram for *m/z* 287.2 in positive ionization mode



Nothing was identified in this ion chromatogram. The ions are of low intensity and probably do not represent substantial products.

Ion chromatogram for *m/z* m/z 311.1 in positive ionization mode



Nothing was identified in this ion chromatogram. The ions are of low intensity and probably do not represent substantial products.

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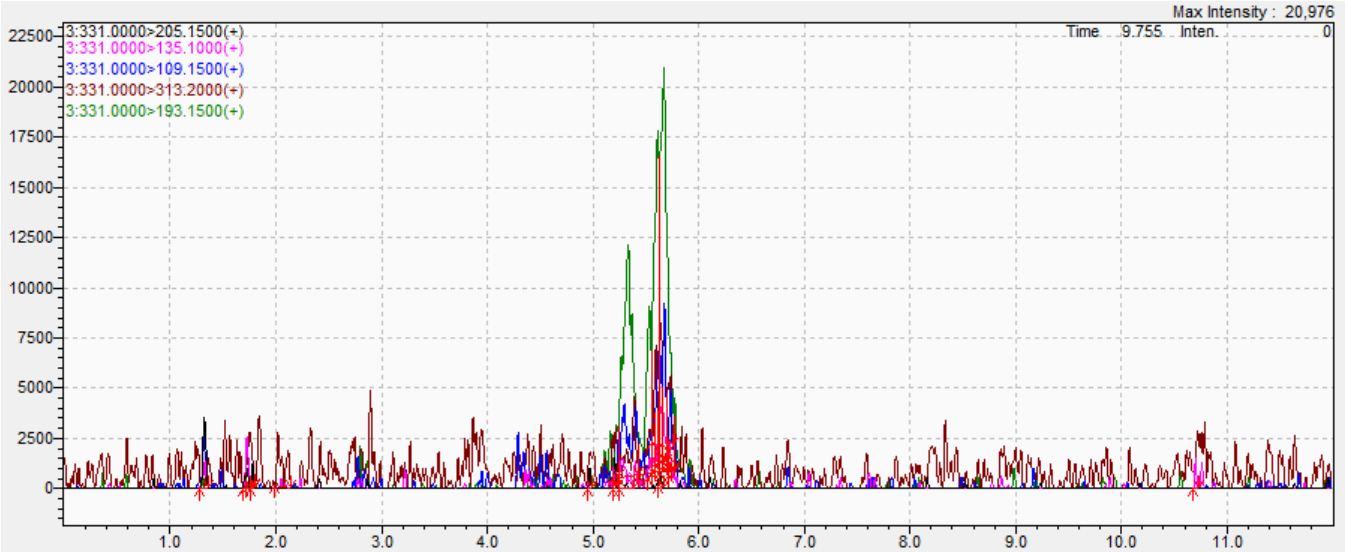
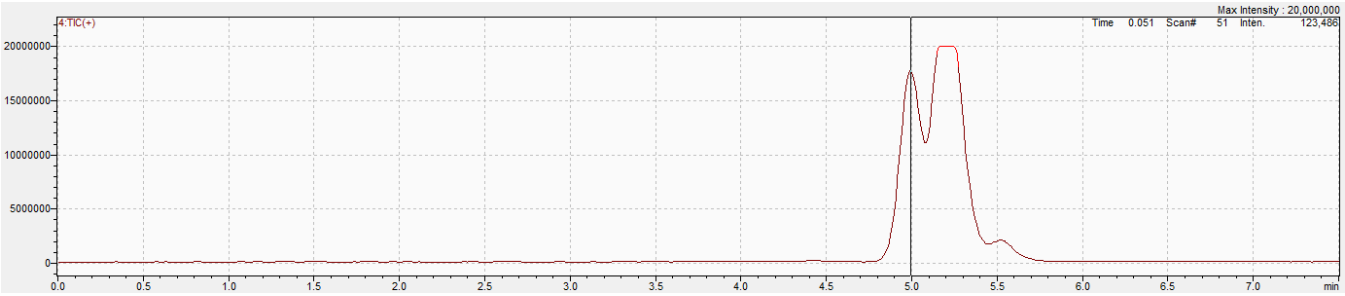
Ion chromatogram for *m/z* 331.0 in positive ionization mode



Nothing was identified in this ion chromatogram. The ions are of low intensity and probably do not represent substantial products.

The selected ion monitoring data for the ion at m/z 317 were collected and are displayed in the following ion chromatograms. The vertical line in the ion chromatogram indicates the time at which the data displayed in the second window were obtained.

The Selected Ion Monitoring (SIM) results for *m/z* 317 in positive ionization mode are shown below. The data at 5.0 minutes were selected for analysis.



The prominent peak at 5.0 min is characterized by *m/z* 317 and the full scan spectrum is characterized by ions at *m/z* 289, 270, and 201.

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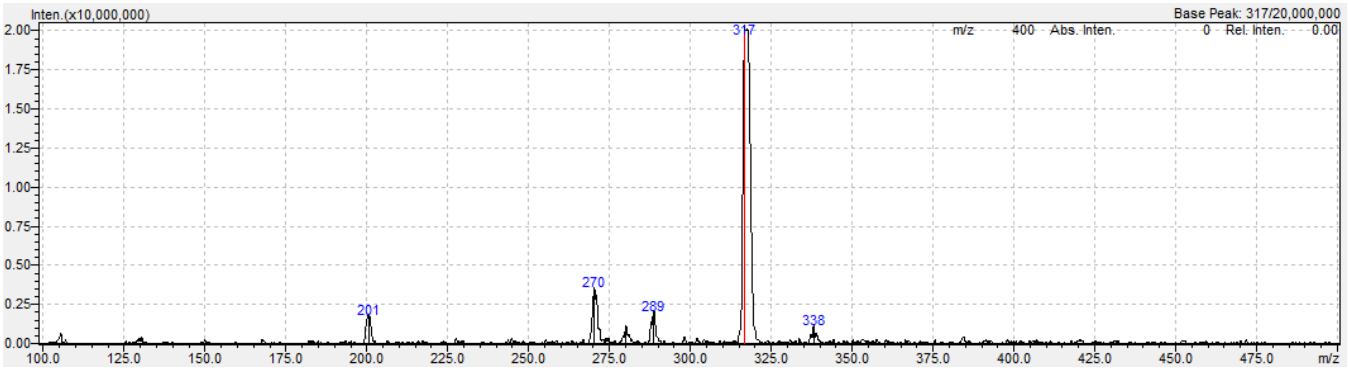
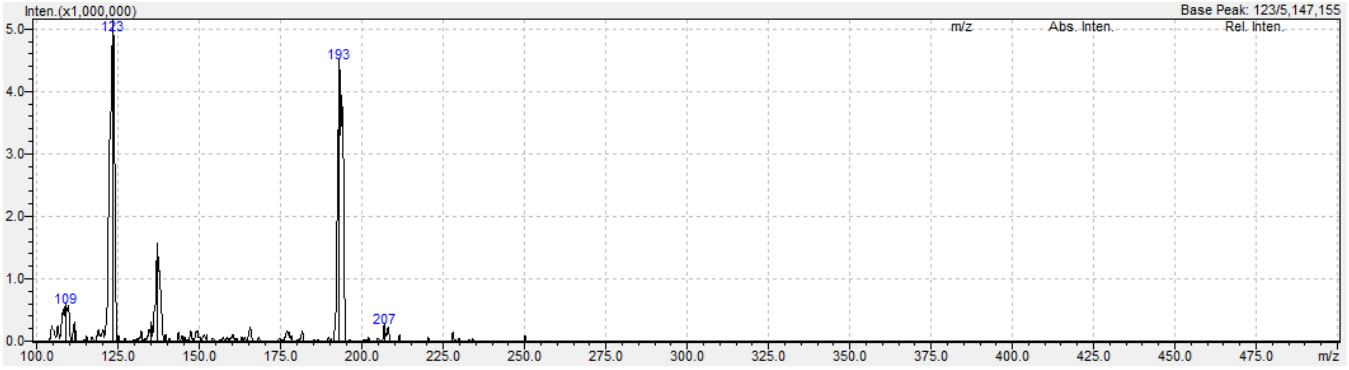
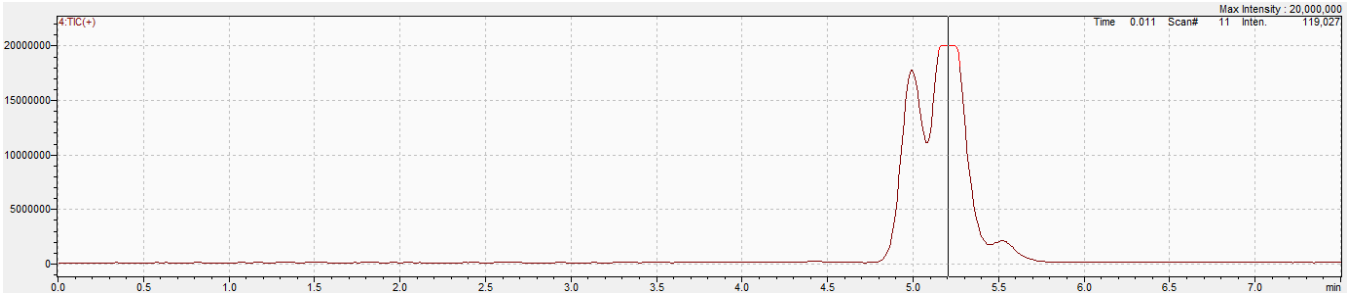


The product ion scan for m/z 317 at 5.0 min is characterized by ions at *m/z* 193 and 123.



SIM of *m/z* 317

The Selected Ion Monitoring (SIM) results for *m/z* 317 in positive ionization mode are shown below. The data at 5.2 minutes were selected for analysis.



The prominent peak at 5.2 min is characterized by *m/z* 317 and the full scan spectrum is characterized by ions at *m/z* 2898 271, and 201.

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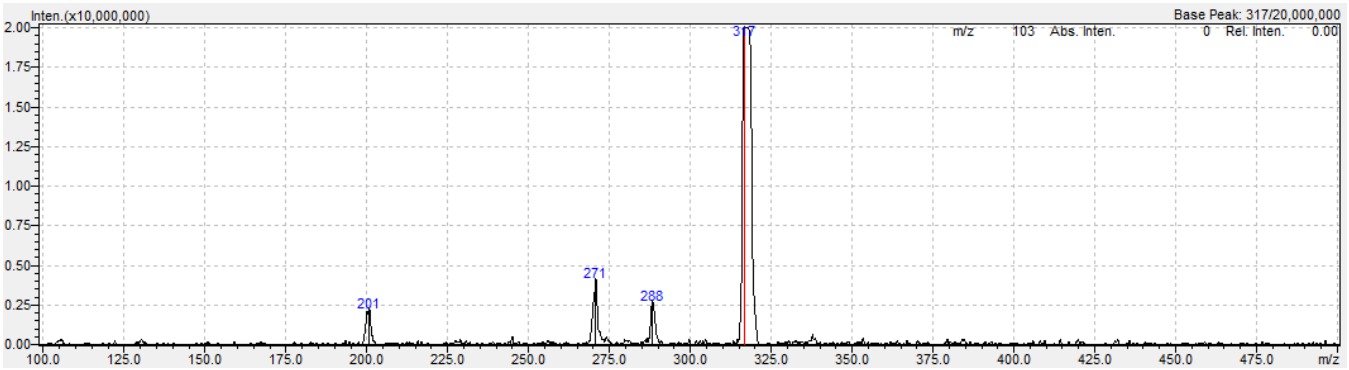

The product ion scan for *m/z* 317 at 5.2 min is characterized by fragment ions at *m/z* 194 and 123.

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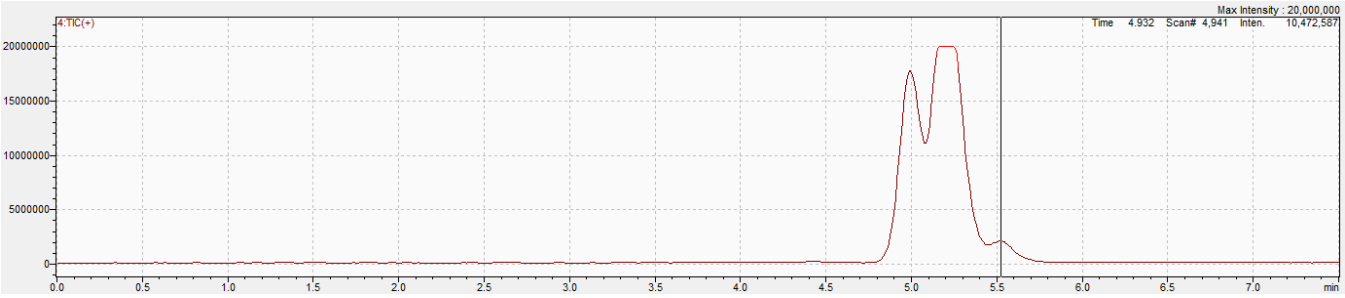
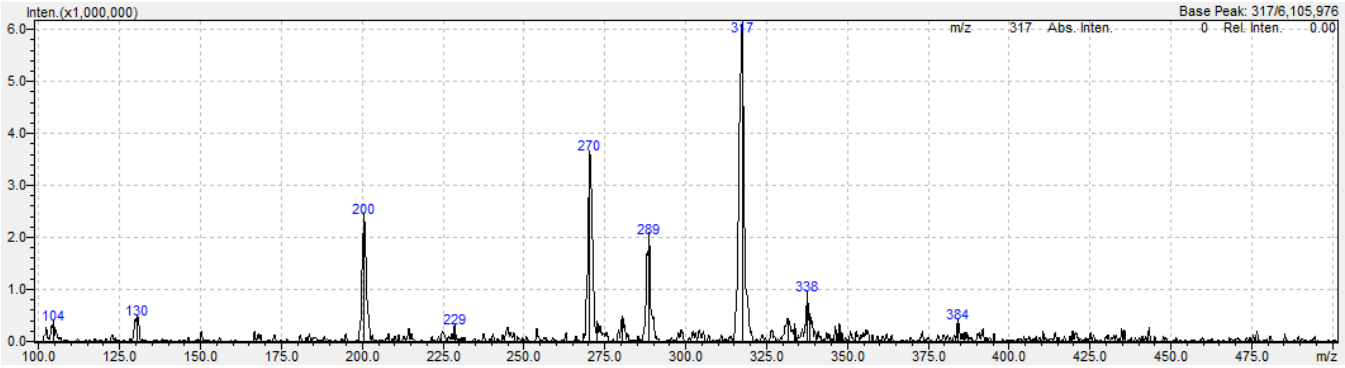
SIM of *m/z* 317

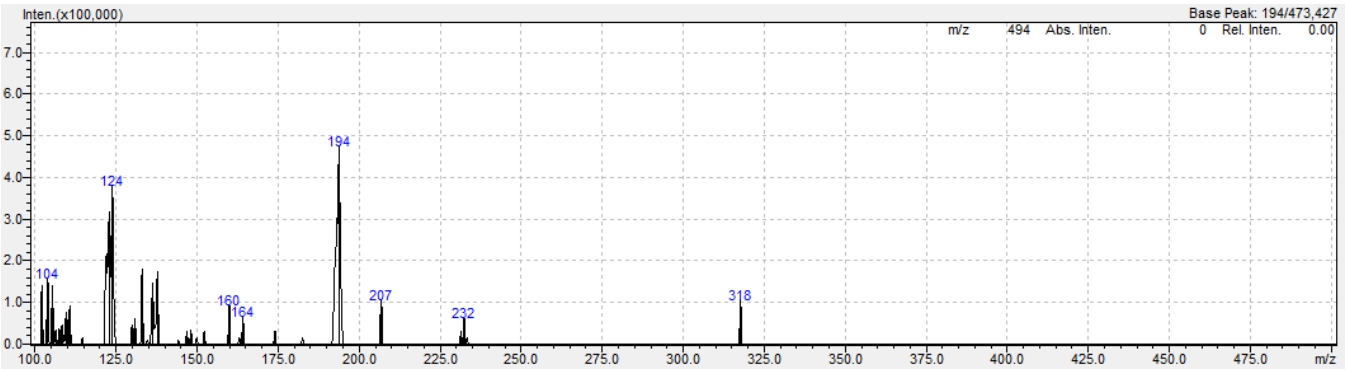
The Selected Ion Monitoring (SIM) results for *m/z* 317 in positive ionization mode are shown below. The data at 5.5 minutes were selected for analysis.



The prominent peak at 5.5 min is characterized by *m/z* 317 and the full scan spectrum is characterized by ions at *m/z* 289, 270, and 200.



The product ion scan for *m/z* 317 at 5.5 min is characterized by fragment ions at *m/z* 194 and 124.



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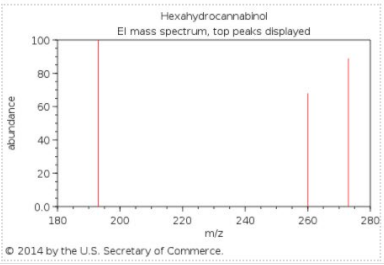
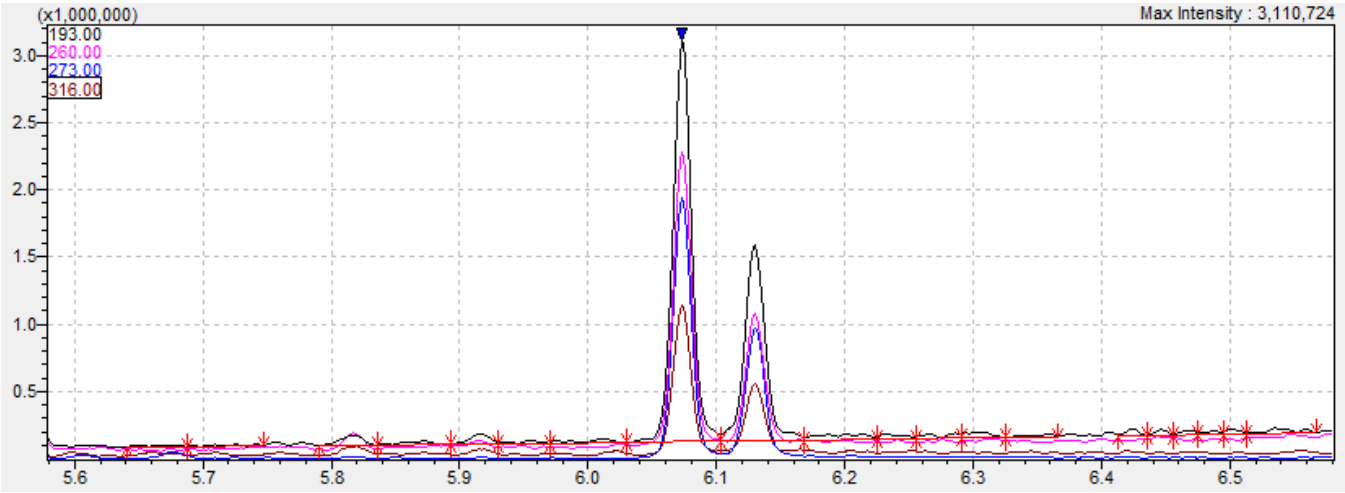
GC-MS/MS Analysis of Sample #2132

The sample was subjected to GC-MS/MS analysis to obtain evidence for the presence of hexahydrocannabinol diastereomers and confirm the identity of the Δ^8-THC.

The GC/MS spectrum of hexahydrocannabinol was obtained from the NIST library and is shown below. Prominent ions are reported at *m/z* 193 (base peak), *m/z* 273, and *m/z* 260.

NIST Number	298729
Library	Replicate library
Total Peaks	155
m/z Top Peak	193
m/z 2nd Highest	273
m/z 3rd Highest	260
Thumbnail	

Those ions characteristic of hexahydrocannabinol were monitored during the GC/MS analysis of Sample 2123. The selected ion chromatogram is shown below and the relative abundances of the ions characteristic of hexahydrocannabinol from the 1st prominent peak at about 6.08 min are shown in the table below.



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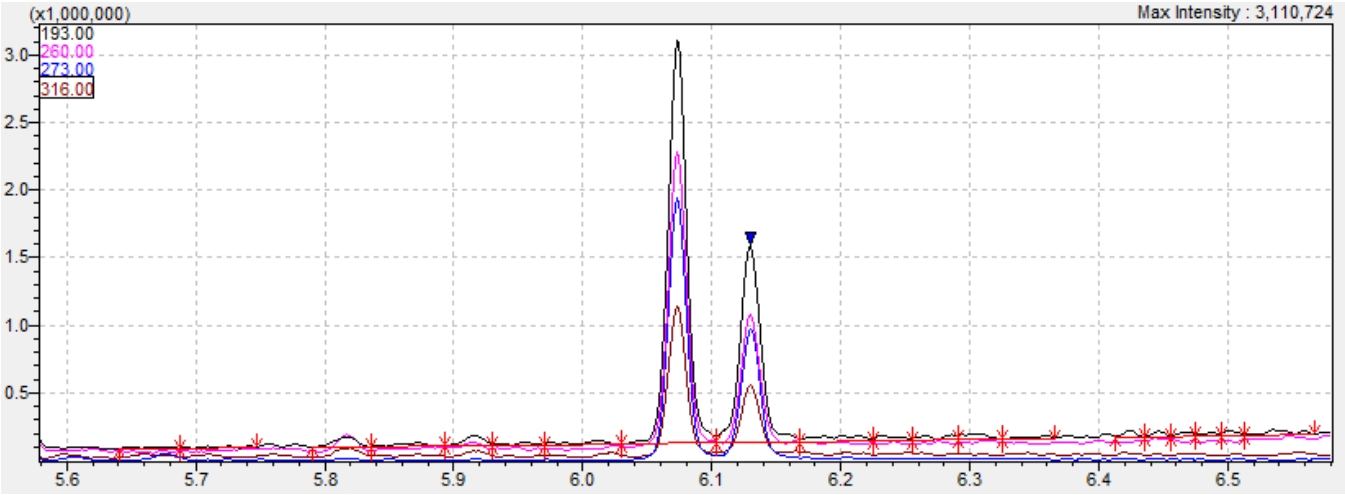
Type	m/z	Intensity	Set %	Act. %	Ref. Band
Target	193.00	3107990	100.00	100.00	---
Ref.Ion1	260.00	2249844	0.00	72.39	30
Ref.Ion2	273.00	2044301	0.00	65.78	30
Ref.Ion3	316.00	846728	0.00	27.24	30
Ref.Ion4					
Ref.Ion5					

The selected ion chromatogram is shown below and the relative abundances of the ions characteristic of hexahydrocannabinol from the 2nd prominent peak at about 6.13 min are shown in the table below.



Type	m/z	Intensity	Set %	Act. %	Ref. Band
Target	193.00	1472511	100.00	100.00	---
Ref.Ion1	260.00	994232	0.00	67.52	30
Ref.Ion2	273.00	955394	0.00	64.88	30
Ref.Ion3	316.00	518360	0.00	35.20	30
Ref.Ion4					
Ref.Ion5					

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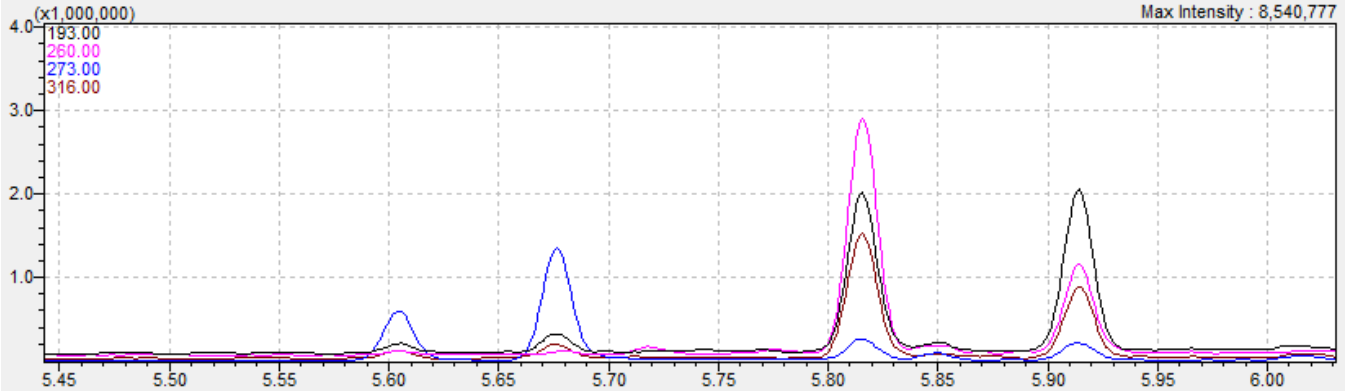
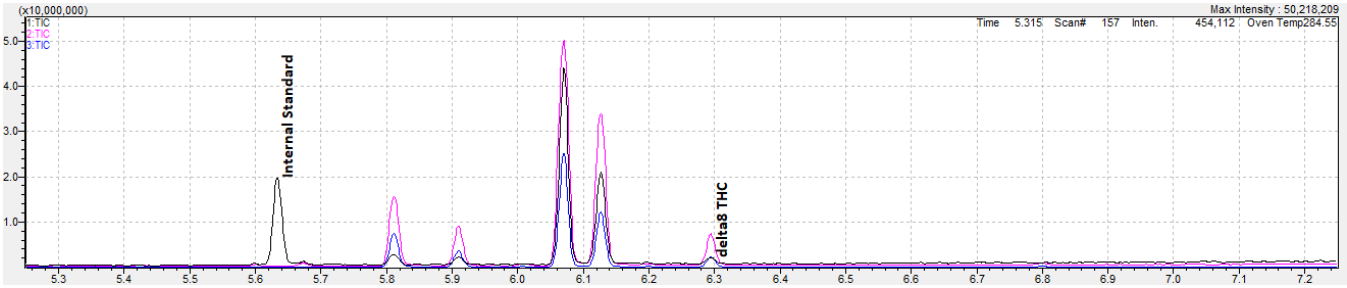
The selected ion chromatograms of the ions characteristic of hexahydrocannabinol from the less abundant peaks before 6.0 min are shown below.



These results indicate with reasonable assurance that at least two and possibly more isomers of hexahydrocannabinol are present in the sample submitted for analysis. Proof of identity would require a reference standard.



The peak at 6.3 minutes was identified as Δ^8-THC. See the following page for confirmation of the identity of Δ^8-THC.

The following is a SIM analysis of a Standards Mix showing ions and retention times for Δ^8-THC and Δ^9-THC. The table below the ion chromatogram reports the relative abundances of the most abundant ion for Δ^8-THC at *m/z* 231, the molecular ion at *m/z* 314, and a fragment ion at *m/z* 299.

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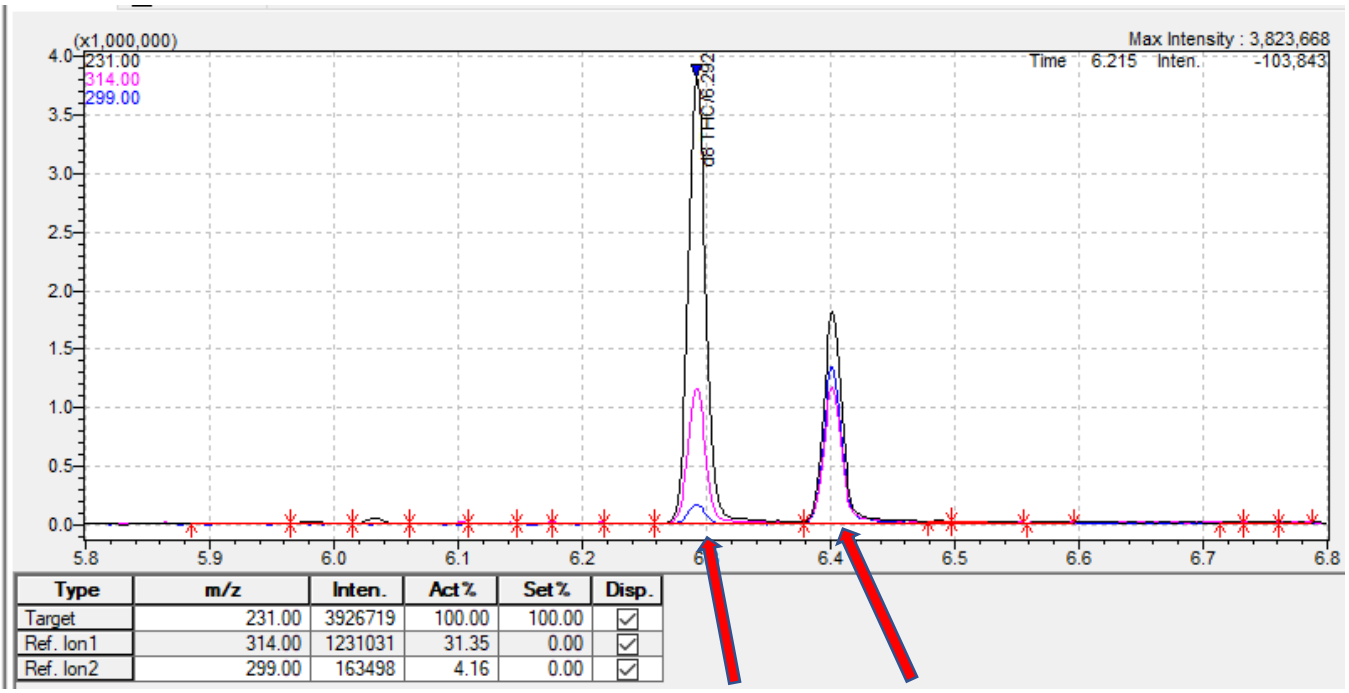
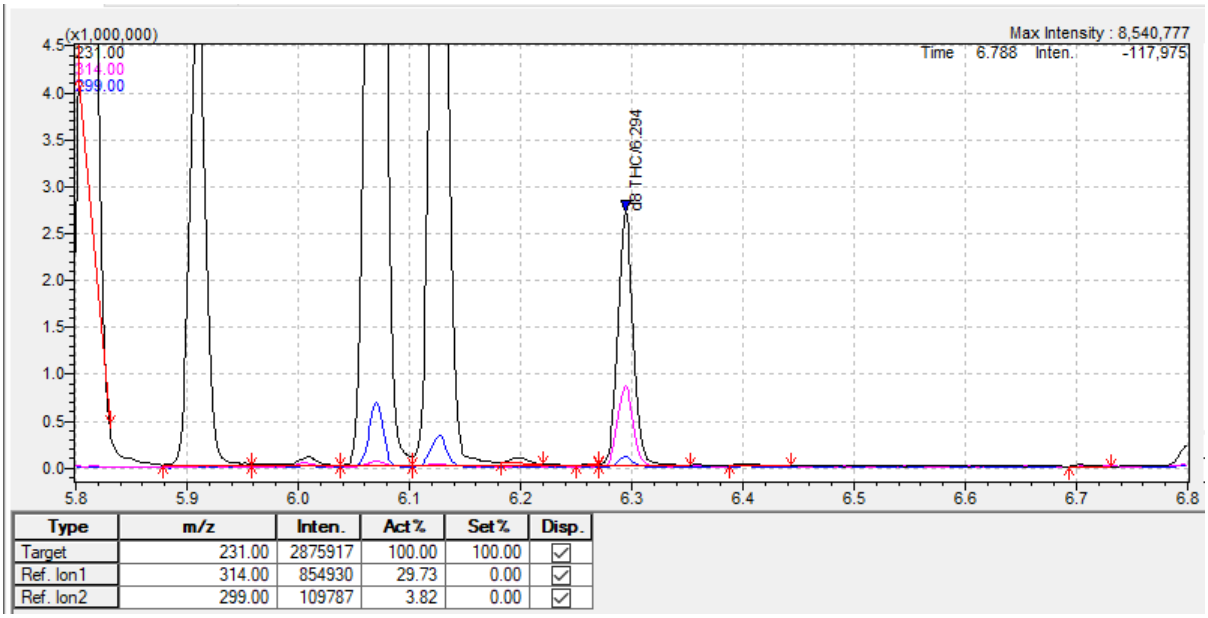


Δ^8 THC Δ^9 THC

The following is a SIM analysis of Sample 2132 showing ions and retention times for Δ^8-THC and Δ^9-THC. The table below the ion chromatogram reports the relative abundances of the most abundant ion for Δ^8-THC at *m/z* 231, the molecular ion at *m/z* 314, and a major fragment ion at *m/z* 299.

SIM analysis of Sample 2132.



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The presence of Δ^8-THC in sample #2132 was confirmed by comparison of the retention time and relative abundances of the qualifier ions with those of a certified reference standard.

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Conclusions

- Sample #2132 contains a small amount of D^8-THC based on HPLC-PDA, LC-MS, and GC-MS data.
- Sample #2132 contains components characterized by greater lipophilicity than D^8-THC based on longer HPLC and LC retention times. The components eluting after D^8-THC are characterized by a pseudomolecular ion at *m/z* 317 based on positive ionization electrospray LC-MS analysis indicating an apparent molecular weight of 316, consistent with that of the hexahydrocannabinol isomers. Analysis of sample #2132 by GC/MS analysis indicated the presence of at least two substances with an apparent molecular mass of 316 and characterized by fragment ions at *m/z* 193, *m/z* 260, and *m/z* 273. These fragment ions are consistent with reported fragment ions of hexahydrocannabinol.
- Confirmation of the presence of hexahydrocannabinol in sample #2132 requires availability of a reference standard.
- Sample #2132 contains no other detectable cannabinoids from our collection of more than forty reference standards.