Determination of Cannabidiol in Various Cannabis Flower Bud Samples using Liquid Chromatography – Mass Spectrometry

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Abstract

This experiment was performed in order to determine the amount of cannabidiol (CBD) present in four cannabis flower bud samples with varying CBD concentrations: *Blue Iguana, Wappa, Mandarin Cookies* and *Miracle 15 x Alien Cookies.* Due to recent legalization, consumption of cannabis products has increased, and can be dangerous for some if not labelled accurately. Concentrations were determined using liquid chromatography - mass spectrometry (LC-MS). LC-MS is an analytical technique that combines liquid chromatography which allows for separation based on polarity of an individual species, and mass spectrometry's ability to identify and quantify specific species. Ions were analyzed by a Q-TOF mass analyzer and detected by a sensitive electron multiplier detector. Signals were recorded and produced analyte peaks as a function of concentration graphs. Five CBD stock solutions were prepared and analyzed to obtain a calibration curve which allowed for determination of CBD present in cannabis samples. CBD was detected in higher quantities than labelled in 2 out of the 4 samples. The concentration of CBD present for *Blue Iguana* was 0.3450 mg/g with a %RSD of 29.07; *Wappa* was 0.35501 mg/g with a %RSD of 2.36; *Mandarin Cookies* was 0.0104 mg/g with a %RSD of 3.49; *Miracle 15 x Alien Cookies* was 0.0941 mg/g with a %RSD of 2.34. The average percent recovery for cannabidiol was 80% for cannabis flower bud samples (n=4). The experimental procedure followed papers by McRae & Melanson (2020) and Romano & Hazekamp (2013).

Introduction

Liquid chromatography – mass spectrometry (LC-MS) is an analytical method used to separate and identify analytes. By combining the effects of liquid chromatography and mass spectrometry, this tool is beneficial for separating isomers that could not be differentiated using other instruments. Liquid chromatography separates individual analytes based on their polarity, by analyzing the stationary and mobile phases. It also monitors the compound separation relating to each analyte's affinities for the mobile phase. Each analyte is then passed through an ionization source in their gas phase after eluting through the column and is then passed to the mass spectrometer. Electrospray ionization (ESI) is used to ionize the sample, by charging the liquid eluent from the LC-MS and dispersing it as a fine spray, leaving molecular ions after evaporation, which are pushed to the mass analyzer. MS has the capability to analyze based on mass to charge ratios and uses a sensitive detector for specific ion determination. The mass analyzer is a quadruple time-of-flight (Q-TOF), which allows ion molecules to be analyzed after separation based on the time it takes to reach the detector; larger ions move slower and smaller ions move faster. This provides sensitive and accurate detection.

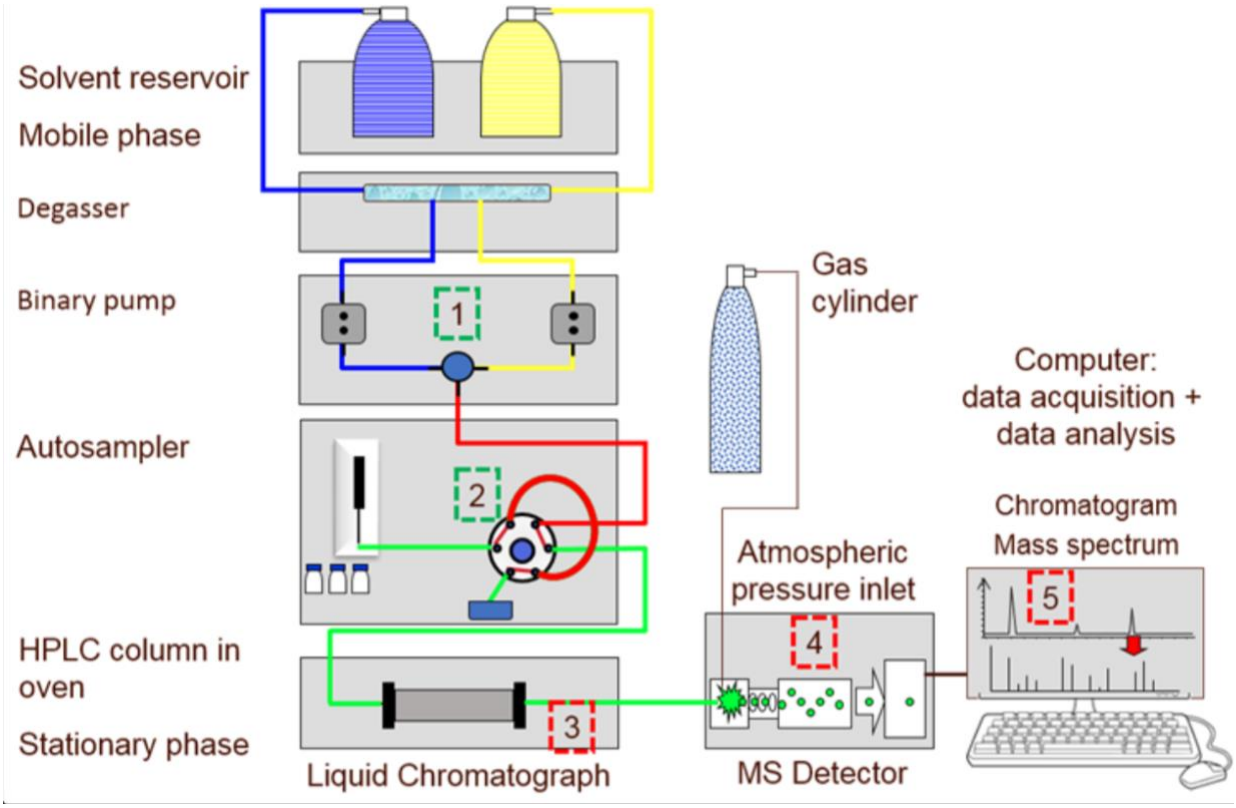

Figure 1. A schematic of an LC-MS instrument (Edwards, 2017).

Cannabis, which was recently legalized in Canada and is widely used across the country, is a product commonly used as a psychoactive. Cannabis comes in many forms, and can be consumed via pills, edible substances, or smoking. Cannabis contains many antioxidant and anti-inflammatory properties; however, it can also cause health issues, like addiction, altered brain development and respiratory issues (Blessing et al., 2015; Volkow et al., 2014). Cannabinoid products typically contain two main active ingredients: cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (Δ^9-THC), the latter being the main psychoactive component. Δ^9-THC can cause dope-dependence, which increases intoxication, causes anxiety, impairment, and psychotic-like symptoms. CBD is typically more calming, and acts as an entourage compound to reduce psychoactive effects (Catenza & Donkor, 2022). Additionally, researchers have hypothesized the ‘entourage effect,’ which states that components of cannabis, like cannabinoids

and terpenes can act together to counteract the psychoactive effect. When terpenes (flavours and aromas) are introduced, positive contribution occurs with the CBD, increasing the terpene and thus increasing human cell signaling pathways. Further understanding of terpenes are required to validate this theory; but may be beneficial to patients suffering with mood disorders, like anxiety and depression (Ferber et al., 2020). Due to this, it is important that CBD and THC content levels are accurate to ensure correct dosing and consumption, and to prevent adverse effects; thus, this experiment is warranted to ensure accurate labeling.

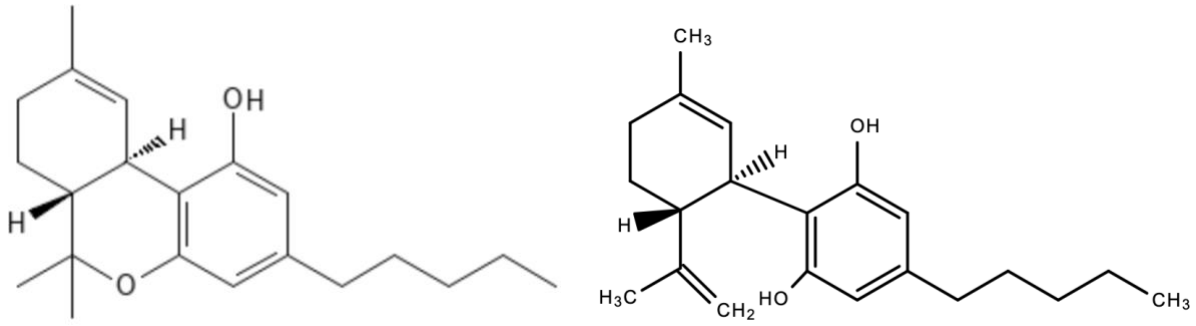

Figure 2. Chemical structure of Δ^9- tetrahydrocannabinol and cannabidiol.

The goal of this project is to identify CBD concentrations accurately and precisely in various cannabis flower samples using liquid chromatography – mass spectrometry, to ensure the labelled amount is correct. McRae & Melanson (2020) address the growing need for standardization of cannabis testing, as current methods are resulting in high variability of results between laboratories. They suggested LC-MS as being the most sensitive and has already been used for detecting cannabinoids in other matrices, like urine and blood. Additionally, Romano & Hazekamp (2013), reviewed multiple extraction methods for cannabis products for both quality and safety issues, reporting that extraction with ethanol and evaporation with a nitrogen blowdown yielded high results. Ethanol does however require additional extraction, as the colour becomes quite dark, and in doing so removes large portions of the cannabinoids and terpenes,

affecting overall concentration. Due to this, methanol was chosen as the solvent for all samples and standards, which has similar yield results to ethanol (Lazarjani et al., 2021). This study will build on this previous research to provide important quality control information and bridge gaps between standardization of testing.

Materials and Methods

Sample and Standard Preparation – Procedure

A stock solution for CBD was prepared by diluting 100 ppm stock solution of CBD in methanol to obtain a 50 ppm stock solution of CBD. From this, five standards were prepared by diluting further with methanol to obtain concentrations of 5, 10, 15, 20 and 25 ppm in a 1.5 mL LC-MS vial.

The 1.0 g samples were prepared by first grinding the flower bud into smaller samples using a grinder obtained from the BC Cannabis Store. Samples were weighed to ensure 1.0 g was accurate. Ground samples were then prepared by diluting them in 25 mL methanol in a 25 mL volumetric flask. All samples and standards were vortexed for approximately 5 minutes each. Samples were filtered through a 0.45 μm syringe filter into a test tube. The solvent in the samples was then evaporated over a boiling water bath, under a constant nitrogen stream. The leftover sample was reconstituted with 4.0 mL methanol. Samples prepared at earlier dates were reconstituted 2.0 mL, and subsequently diluted in 1.5 ml increments of methanol as required due to evaporation. 500 μL of the sample was diluted with 500 μL of 18 MOhm water in LC-MS sample vials to prevent dark sample colour interfering with the analysis. All samples and standards were transferred to LC-MS sample vials and ran using the parameters in Table 2. Samples were run in triplicate.

For precision studies, three standards were prepared by diluting a 1000 ppm stock to 50 ppm with methanol. 5, 15 and 25 ppm standards were prepared by diluting with methanol to a total of 1.0 mL, in the LC-MS vials. They were run in triplicate over three days to ensure precision.

To obtain accurate concentrations of samples, the samples were spiked with 20 ppm of the CBD stock solution, prepared by diluting a 50 ppm CBD stock solution in methanol. 0.5 ml of sample was diluted with 0.5 ml of spiked solution. 0.5 ml of sample and 0.5ml 18 MOhm water was ran alongside for accuracy. Both were run in triplicate on the LC-MS using parameters in Table 1.

Chemicals and solvents

50 ppm CBD Stock, Methanol, Nitrogen, 99.7% Acetonitrile, 0.3% Formic Acid, 74.7% 18 MOhm Water, 25% Methanol, 0.3% Formic Acid

Sample Information

Table 1. Names and data of samples obtained from various BC Cannabis stores.

Sample ID	**LC-MS Sample ID**	**Labelled THC Concentration (mg/g)**	**Labelled CBD Concentration (mg/g)**	**Company**
Blue Iguana	BI	3.30	< 0.10	Weed Me
Wappa	RW	15.0	0.00	Redecan
Mandarin Cookies	MC	3.80	< 0.10	Weed Me
Miracle 15 x Alien Cookies	MA	5.19	< 0.50	Holy Mountain

Instrument Information

Agilent Technologies G530 Accurate-Mass Q-TOF LC/S 1200 series, internal diameter of 1.8 µm and column width/length of 2.1 x 100 mm. Ionization Source: ESI+.

Table 2. Instrumental parameters of the Agilent Technologies Accurate-Mass Q-TOF LC/S 1200 series.

VCap:	3000 V	**Flow Rate:**	0.5 mL/min
Fragementor:	60.0 V	**Injection Size:**	5.0 µL
Gas Temperature:	350°C	**Column Temperature:**	30°C
Drying Gas:	8.0 L/min	**Solvent A:**	74.4% 18 MOhm water, 25% Methanol, 0.3% Formic Acid
Nebulizer:	15 psig	**Solvent B:**	99.7% Acetonitrile, 0.3% Formic Acid
Sheath Gas Temperature:	325°C	**Elution Gradient:**	100% A to 100% B in 4 minutes, hold at 100% B for 8 minutes. After 30 s, 100% A, hold for 3 minutes
Sheath Gas Flow:	8.0 L/min	**Stop Time:**	5 mins
Acquisition:	100 – 500 m/z		

Results and Discussion

Optimizing Analysis Conditions

Based on previous research by Meng et al. (2018), it was recommended to use an Agilent Eclipse Plus C18 column, which was used in this study to separated cannabidiol from the cannabis samples. The mobile phase consisted of solvent A: 74.4% 18 MOhm water, 25% Methanol, 0.3% Formic Acid and solvent B: 99.7% Acetonitrile, 0.3% Formic Acid. Based on previous methods, run time was set for 15 minutes; however, most peaks came out at a retention time of around 7-8 minutes or 11 minutes, depending on the sample. By using this method, a calibration curve was successfully generated, ranging in concentration from 5 ppm to 25 ppm, as seen in Figure 3. The chromatogram in Figure 4 shows the increasing peak areas of the standards relative to the cannabidiol concentrations.

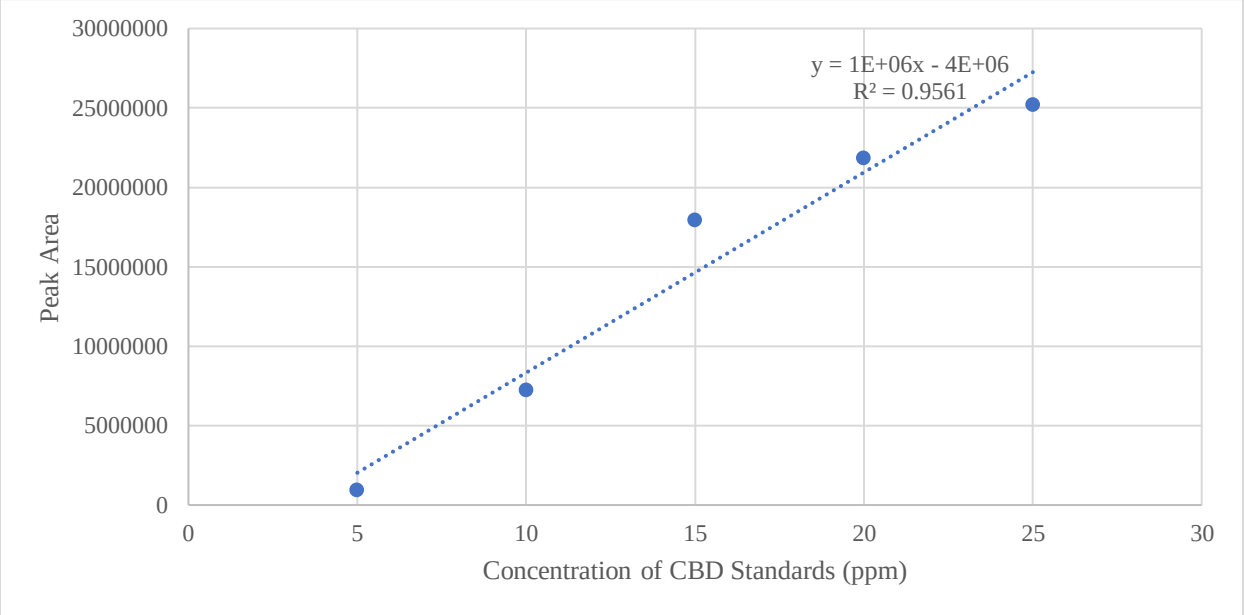

Figure 3. Calibration curve of cannabidiol standards (n=5).

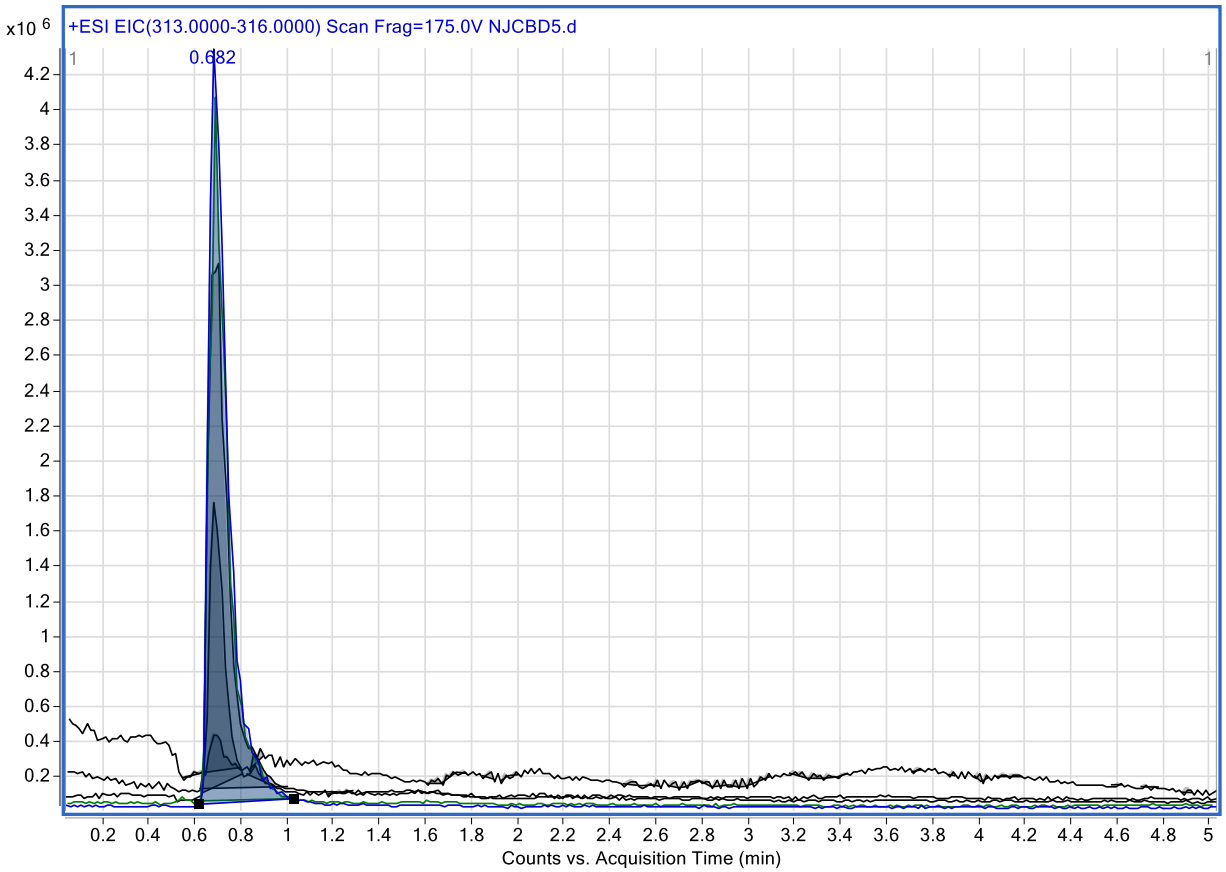

Figure 4. Chromatogram of overlaid cannabidiol standards of 5 ppm to 25 ppm (n=5).

Analysis of Cannabis Flower Samples

Cannabidiol was found to be in higher concentration than the labelled amount in 2 of the 4 samples analyzed. Most notably, the concentration found in *Wappa* was 0.3551 mg/g with a percent relative standard deviation (%RSD) of 2.36%, compared to the labelled amount of 0.00 mg/g. *Blue Iguana* was found to have 0.2 mg/g more cannabidiol than labelled, with 0.3450 mg/g and a %RSD of 29.07%. This high %RSD was most likely due to run 1 eluting later than runs 2 and 3. *Mandarin Cookies* and *Miracle 15 x Alien Cookies* showed lower amounts than labelled, only having 0.0941 mg/g and a %RSD of 3.49% and 0.010 mg/g with a %RSD of 2.34% present, respectively; however, this could be due to detection issues. The spiked samples showed similar cannabidiol concentrations. *Wappa* and *Blue Iguana* showed a higher concentration than that of the plain samples, with 0.3840 mg/g and 0.3360 mg/g, respectively.

Mandarin Cookies showed a much lower concentration that was detected, with 0.056 mg/g. Compared to the amount calculated in the plain sample, there may have been contaminants, or the sample may have degraded. *Miracle 15 x Alien Cookies* was found to have 0.096 mg/g, which is much higher when compared to the plain sample. Similar to the aforementioned sample, there may have been contaminants, or the sample may have degraded overtime. The percent recovery for *Wappa* and *Blue Iguana*, was calculated to be 120% and 105%, respectively. *Miracle 15 x Alien Cookies* had a percent recovery of 60%, which is lower than anticipated, but could be due to low concentration present in the solution. The percent recovery of *Mandarin Cookies* was the lowest, with 35%, again due to low concentration present in sample. See Table 3 and 4 for values below. See Appendix for chromatogram results.

Due to the dark colour of the samples, the LC-MS had issues detecting the samples, thus, further dilutions with deionized water in the LC-MS vials had to be done to ensure accurate detection. In doing so, this may have lowered the concentrations of the samples. Future work could use a hydrocarbon extraction to remove the dark pigment while maintaining the cannabidiol concentration prior to analysis (Lazarjani et al., 2021).

Table 3. Determined concentration of CBD found in cannabis flower bud samples.

Samples	**CBD Concentration in Samples (ppm)**	**Concentration in (mg/g)**	**Labelled CBD Concentration (mg/g)**
Blue Iguana (BI)	21.565	0.3451	< 0.100
Wappa (RW)	22.193	0.3551	0.000
Mandarin Cookies (MC)	1.3017	0.0104	< 0.100
Miracle 15 x Alien Cookies (MA)	11.761	0.0941	< 0.500

Table 4. Determined cannabidiol concentration by interpolation of spiked samples.

Samples	**Interpolated CBD Concentration in Spiked Samples (ppm)**	**Concentration (mg/g)**	**Percent Recovery (%)**
Blue Iguana (BI)	21.00	0.3360	105
Wappa (RW)	24.00	0.3840	120
Mandarin Cookies (MC)	7.000	0.0560	35
Miracle 15 x Alien Cookies (MA)	12.00	0.0960	60

Table 5. Retention time for samples and spiked samples.

Spike Data – Retention Time					
		BI	**RW**	**MC**	**MA**
Sample					
Runs	1	7.421	7.489	10.613	7.539
	2	7.453	7.506	10.774	7.630
	3	7.358	7.431	10.757	7.524
Sample + Spike					
Runs	1	8.343	7.545	8.510	8.507
	2	7.969	7.663	9.937	8.611
	3	7.897	7.636	10.664	8.349

Table 6. Peak area for samples and spiked samples

Spike Data - Peak area					
		BI	**RW**	**MC**	**MA**
Sample					
Runs	1	33310027	5790454	5876830	24070654
	2	34312132	20009972	6359488	16606488
	3	20153932	19785515	653913	16157390
Sample + Spike					
Runs	1	18602806	29982757	1005804	9209132
	2	25198746	27895741	1502169	9039307
	3	26027875	29948972	1876325	10091993

Method Validation

A calibration curve was generated for cannabidiol concentrations ranging from 5 ppm to 25 ppm, with a high coefficient of linearity, R^2 of 0.9561. The percent relative standard deviation (%RSD) for peak area for intraday precision for 5, 15 and 25 ppm was 6.46%, 7.33%, and 4.56%, respectively. The average %RSD for peak area for interday precision for 5, 15 and 25 ppm was 11.59%, 5.97%, and 4.39% respectively. Comparably, %RSD for retention time of 5 , 15 and 25 ppm for intraday precision was 0.819%, 0.448% and 0.652%, respectively. The average %RSD for retention time for interday precision for 5, 15 and 25 ppm was found to be 2.201%, 0.530% and 0.554%, respectively (see Table 9 and 10). These results indicate the method is reliable and sensitive to detect low concentrations of cannabidiol. The average percent recovery for cannabidiol was 80% for cannabis flower bud samples (n=4). See Appendix for complete results.

Table 7. Average interday precision results. See Appendix for all values.

Concentration (ppm)	Peak Area	Retention Time (min)
Day 1:		
5	2590267.000	6.502
15	10744552.00	6.479
25	13327466.33	6.470
Day 2:		
5	4737889.333	6.484
15	12548476.00	6.491
25	14190643.00	6.492
Day 3:		
5	6133940.30	6.695
15	14105070.67	6.816
25	22894699.67	6.818

Table 8. Average intraday precision results. See Appendix for all values.

Concentration (ppm)	Peak Area	Retention Time (min)
5	4569642	6.545
15	13037669	6.480
25	14929170	6.464
5	5091234	6.459
15	11486059	6.469
25	13920428	6.541
5	4552792	6.448
15	13121700	6.524
25	13722331	6.472

Table 9. Average interday precision results for percent relative standard deviation for retention time and peak area.

Concentration (ppm)	%RSD for Retention Time	%RSD for Peak Area
5	2.201	11.59
15	0.530	5.97
25	0.554	4.39
Average	1.095	7.31

Table 10. Average intraday precision results for percent relative standard deviation for retention time and peak area.

Concentration (ppm)	%RSD for Retention Time	%RSD for Peak Area
5	0.819	6.46
15	0.448	7.33
25	0.652	4.56
Average	0.639	3.96

Future work

This research was able to identify the amount of cannabidiol (CBD) present in cannabis flower bud samples to compare to their labelled content. In the future, multiple trials over several days for the spiked samples could be done to ensure more accurate concentrations of samples.

Additionally, flower samples with higher concentrations of CBD could be used, as it might allow for more accurate detection as most of the samples had very low CBD content. Furthermore, the concentrations for used for intraday precision could be increased to ensure more accurate readings, as the lower concentrations (5 ppm) had higher relative standard deviations due to low concentration. As limit of detection (LOD) and quantification (LOQ) was unable to be determined due to low obtained values, future work could be done to determine both LOD and LOQ to further validate the method. Future work could also investigate the entourage effect, especially with flower samples as they often combine various flavor and aroma profiles to produce the best product. Further investigation into this would be able to tailor cannabis strains to better target medical issues, like mental and physical health, and give researchers a better understanding of cannabis interactions, while lowering side effects.

Conclusion

This study allowed for determination of cannabidiol concentration in various cannabis flower bud samples, using liquid chromatography – mass spectrometry. The determined concentration of cannabidiol was found to be higher in 2 of the 4 samples. The concentration of CBD present for *Blue Iguana* was 0.3450 ± 0.0618 mg/g; *Wappa* was 0.35501 ± 0.0445 mg/g; *Mandarin Cookies* was 0.0104 ± 0.0258 mg/g; *Miracle 15 x Alien Cookies* was 0.0941 ± 0.0243 mg/g. The precision of this study was acceptable with percent relative standard deviation ranging from 2.34% to 17.92%. The average percent recovery for cannabidiol was 80% for cannabis flower bud samples (n=4).

Acknowledgements

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Appendix

Interday Precision

Concentration (ppm)		Peak Area	Retention Time (min)
5			
	1	2161496	6.533
	2	3147142	6.496
	3	2462163	6.478
15			
	1	10274178	6.450
	2	11310020	6.505
	3	10649458	6.481
25			
	1	12863641	6.459
	2	14093709	6.472
	3	13025049	6.480
Concentration (ppm)		Peak Area	Retention Time (min)
5			
	1	4569642	6.545
	2	5091234	6.459
	3	4552792	6.448
15			
	1	13037669	6.480
	2	11486059	6.469
	3	13121700	6.524
25			
	1	14929170	6.464
	2	13920428	6.541
	3	13722331	6.472
Concentration (ppm)		Peak Area	Retention Time (min)
5			
	1	4718343	6.282
	2	14498606	6.872
	3	23489878	6.773
15			
	1	6585174	6.927
	2	13179239	6.785
	3	23238634	6.798
25			
	1	7098304	6.875
	2	14637367	6.790
	3	21955587	6.883

Intraday Precision

Concentration (ppm)	Peak Area	Retention Time (min)
5	4569642	6.545
15	13037669	6.480
25	14929170	6.464
5	5091234	6.459
15	11486059	6.469
25	13920428	6.541
5	4552792	6.448
15	13121700	6.524
25	13722331	6.472

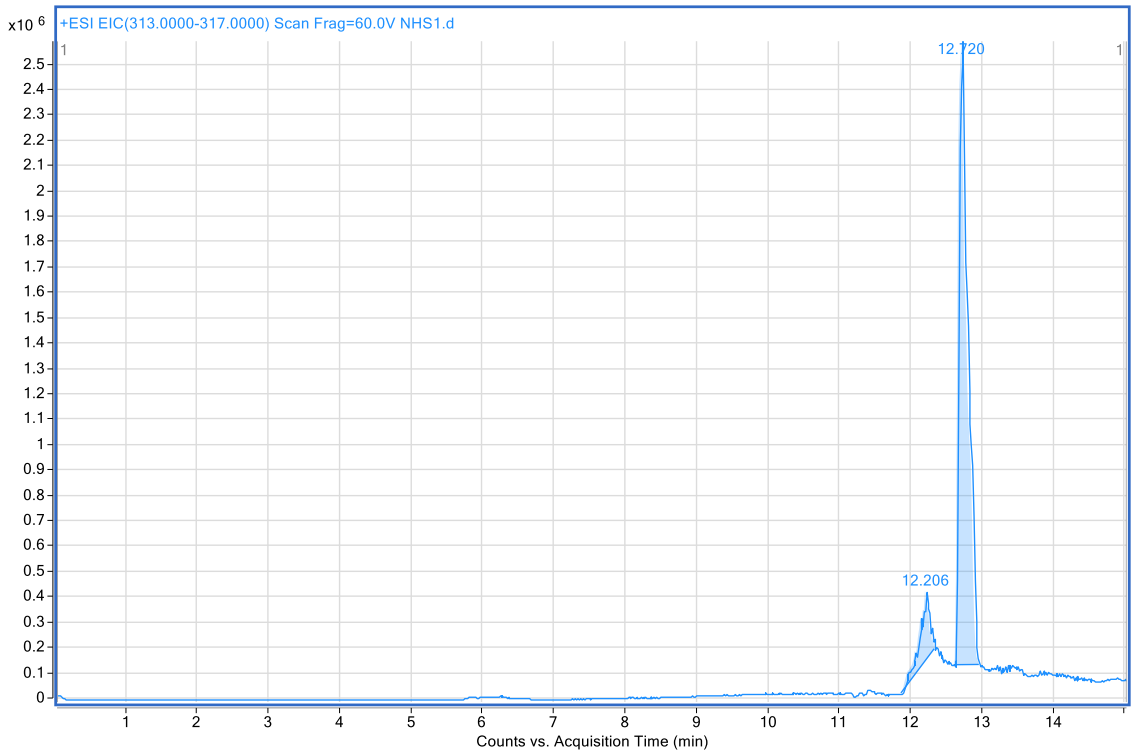
%RSD for retention time and peak area of interday and intraday precision studies

Concentration	%RSD RT	%RSD PA
Day 1:		
5	0.431	19.5020784
15	0.426	4.88086991
25	0.164	5.01577056
Day 2:		
5	0.819	6.46113477
15	0.448	7.33984694
25	0.652	4.56080273
Day 3:		
5	5.352	20.4192152
15	0.717	5.70567824
25	0.846	3.59445666

****NOTE: Day 2 results correspond to intraday precision results**

Chromatogram of samples:

BI Run 1



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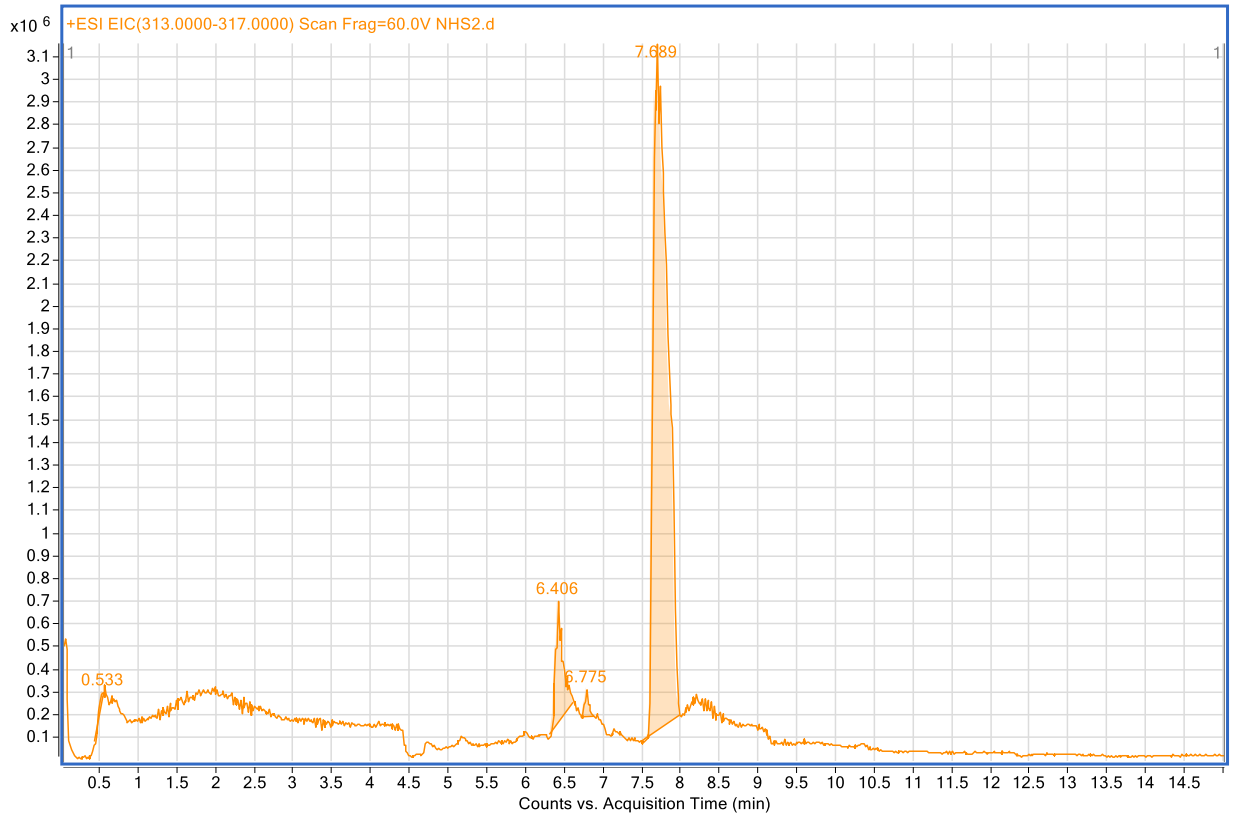

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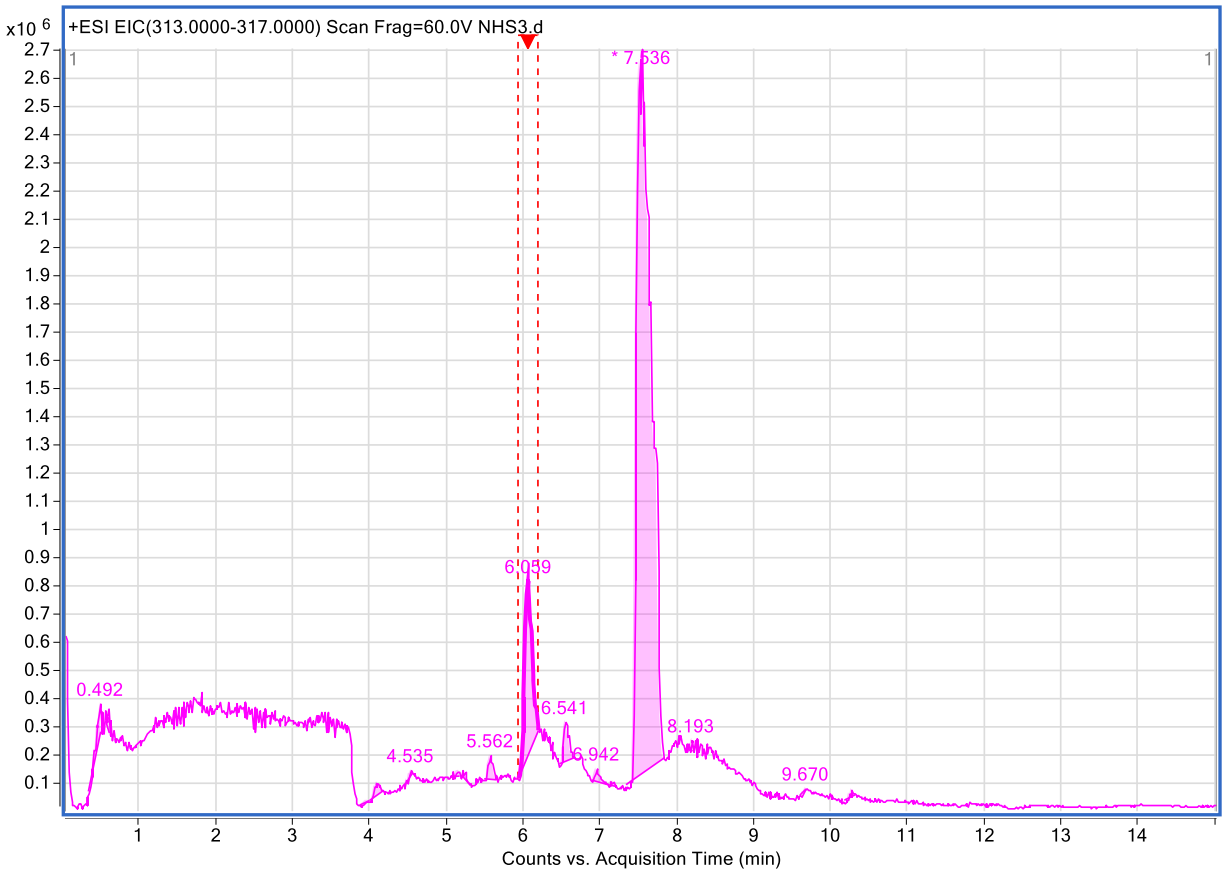
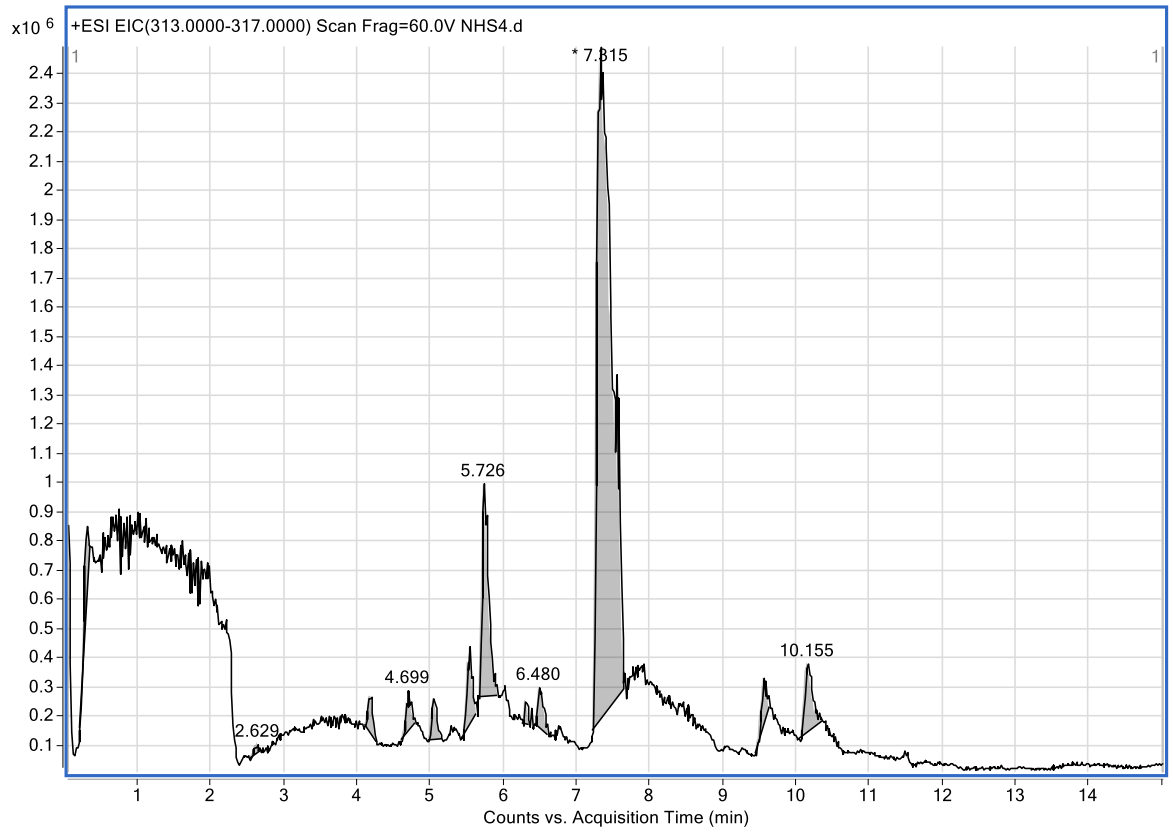
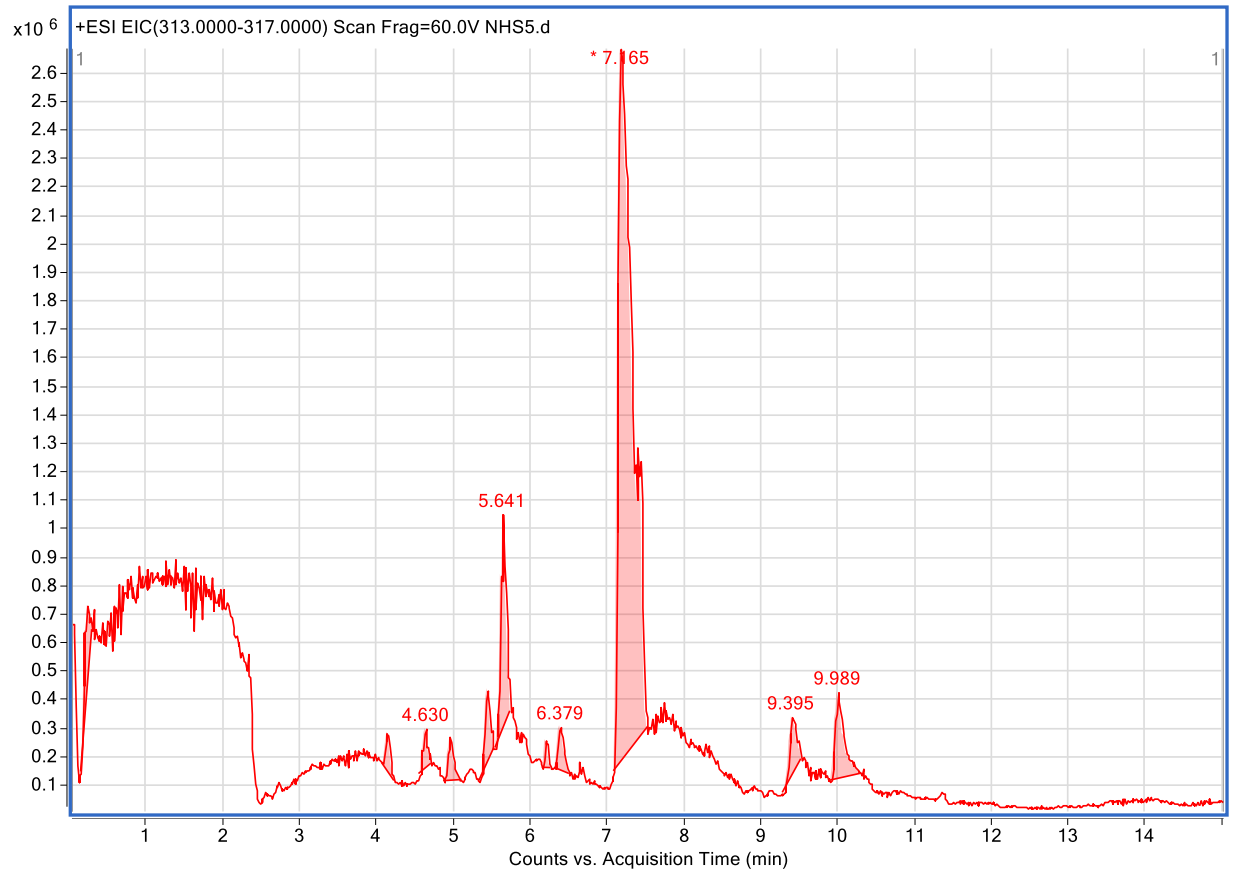

x10 6
+ESI EIC(313.0000-317.0000) Scan Frag=60.0V NHS3.d
* 7.536
6.059
0.492
4.535
5.562
6.541
6.942
8.193
9.670
Counts vs. Acquisition Time (min)

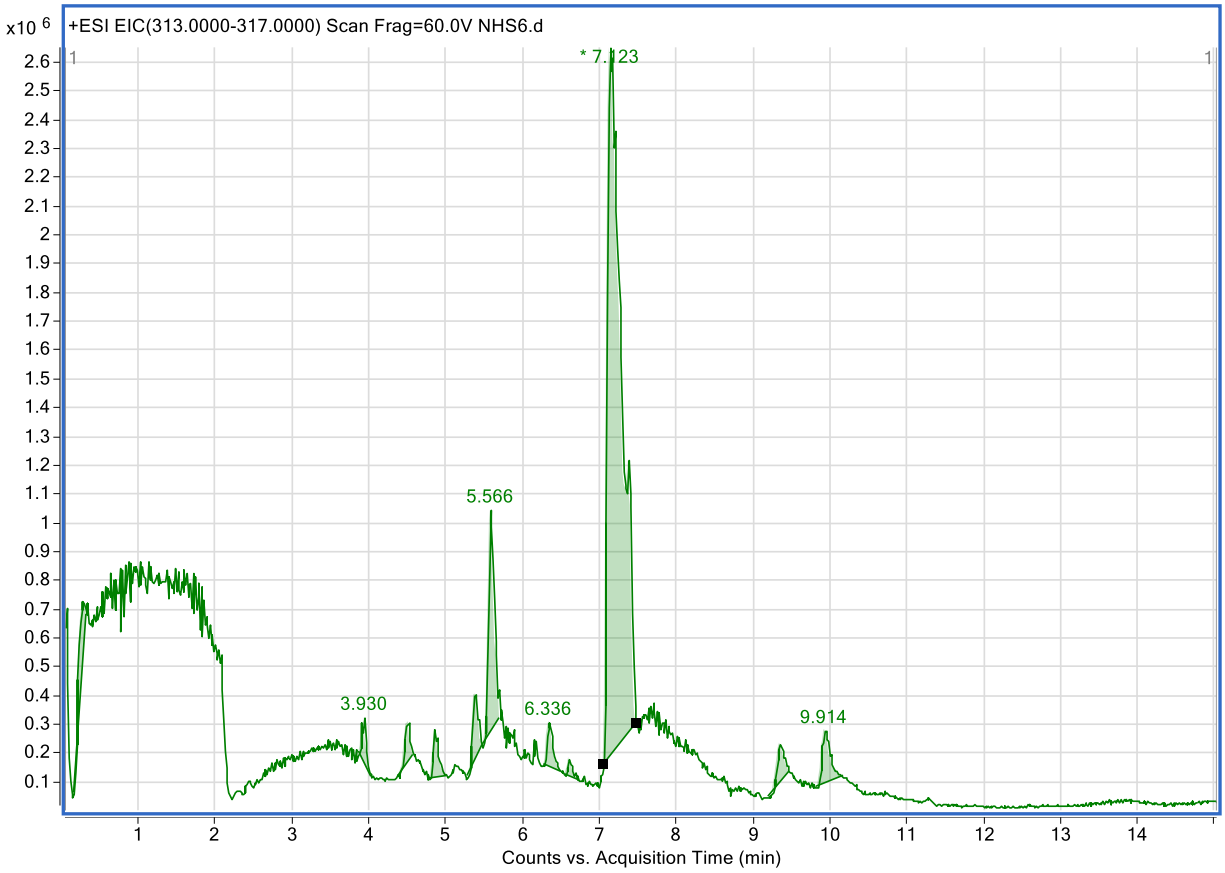
RW Run 1


x10 6
+ESI EIC(313.0000-317.0000) Scan Frag=60.0V NHS4.d
* 7.315
5.726
4.699
6.480
2.629
10.155
Counts vs. Acquisition Time (min)

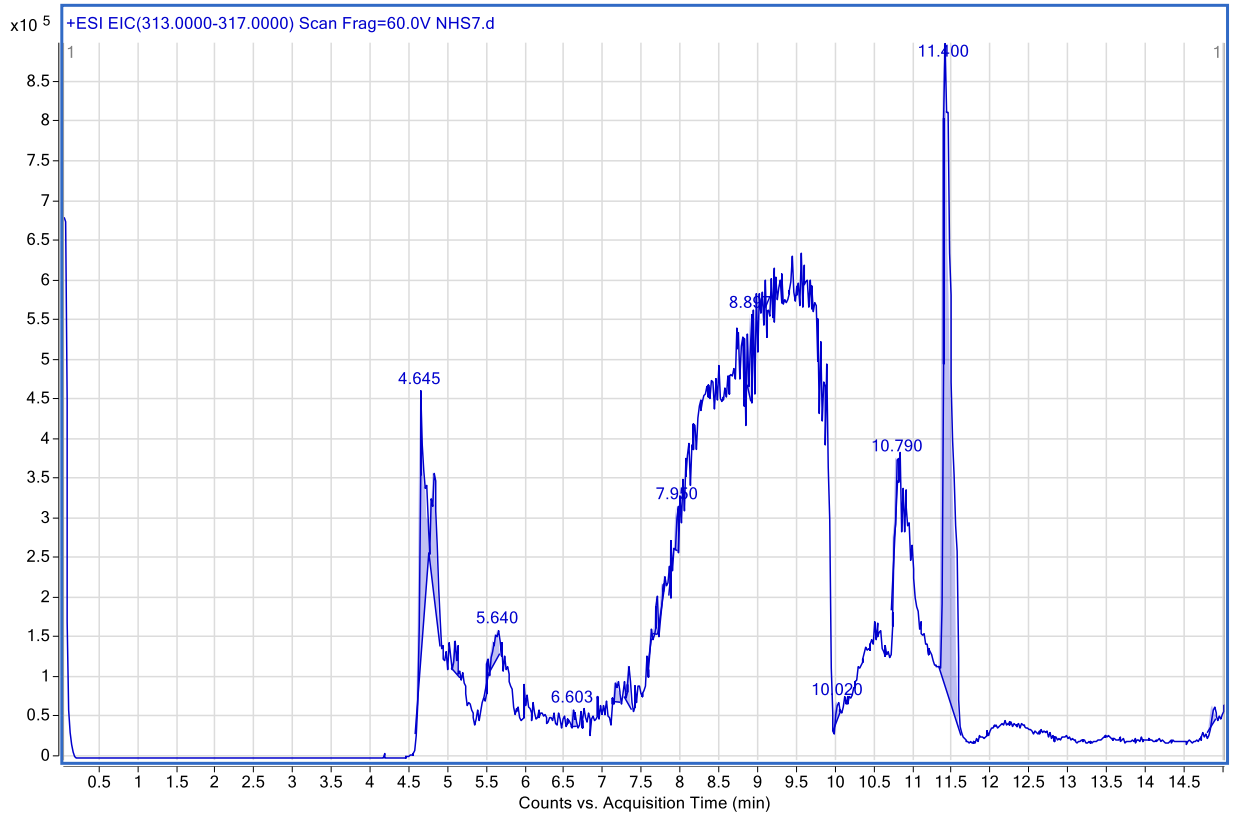
RW Run 2


x10 6
+ESI EIC(313.0000-317.0000) Scan Frag=60.0V NHS5.d
* 7.165
5.641
4.630
6.379
9.395
9.989
Counts vs. Acquisition Time (min)

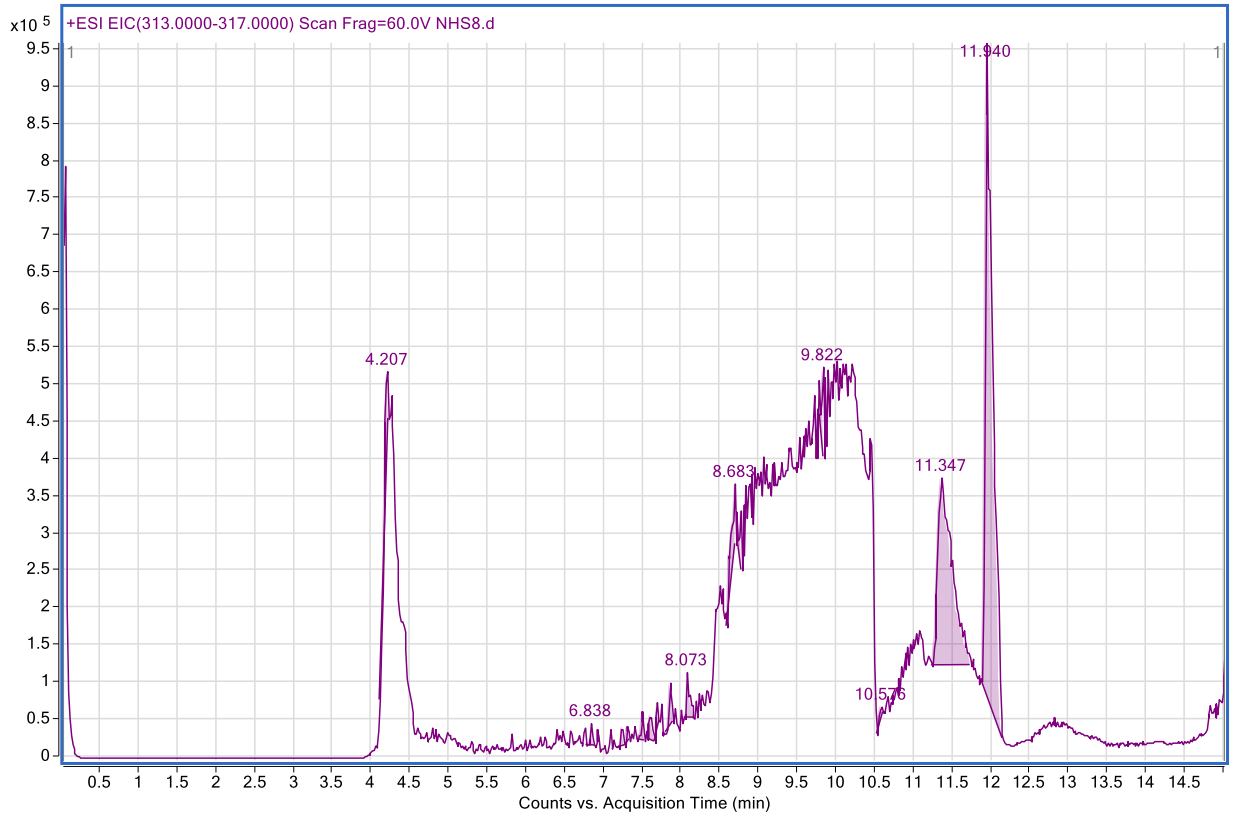
RW Run 3


x10 6
+ESI EIC(313.0000-317.0000) Scan Frag=60.0V NHS6.d
* 7.123
5.566
3.930
6.336
9.914
Counts vs. Acquisition Time (min)

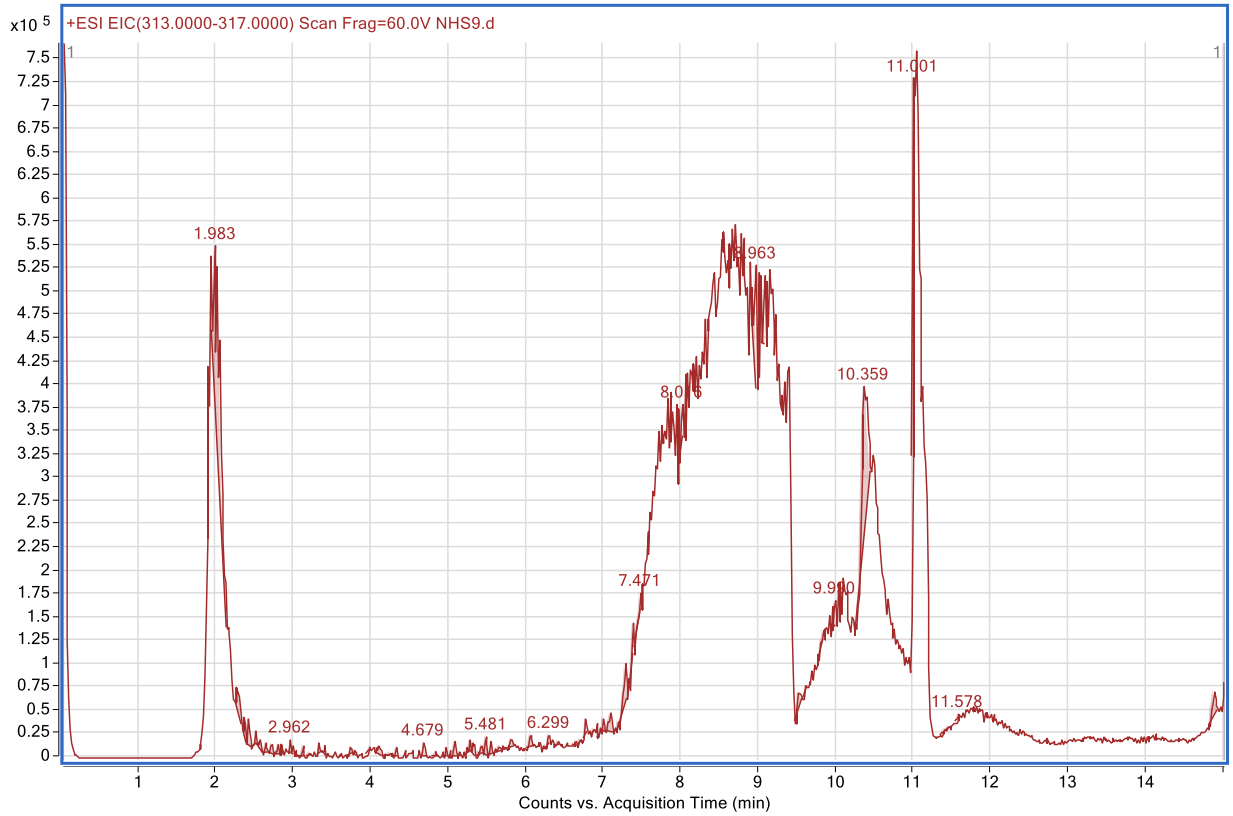
MC Run 1



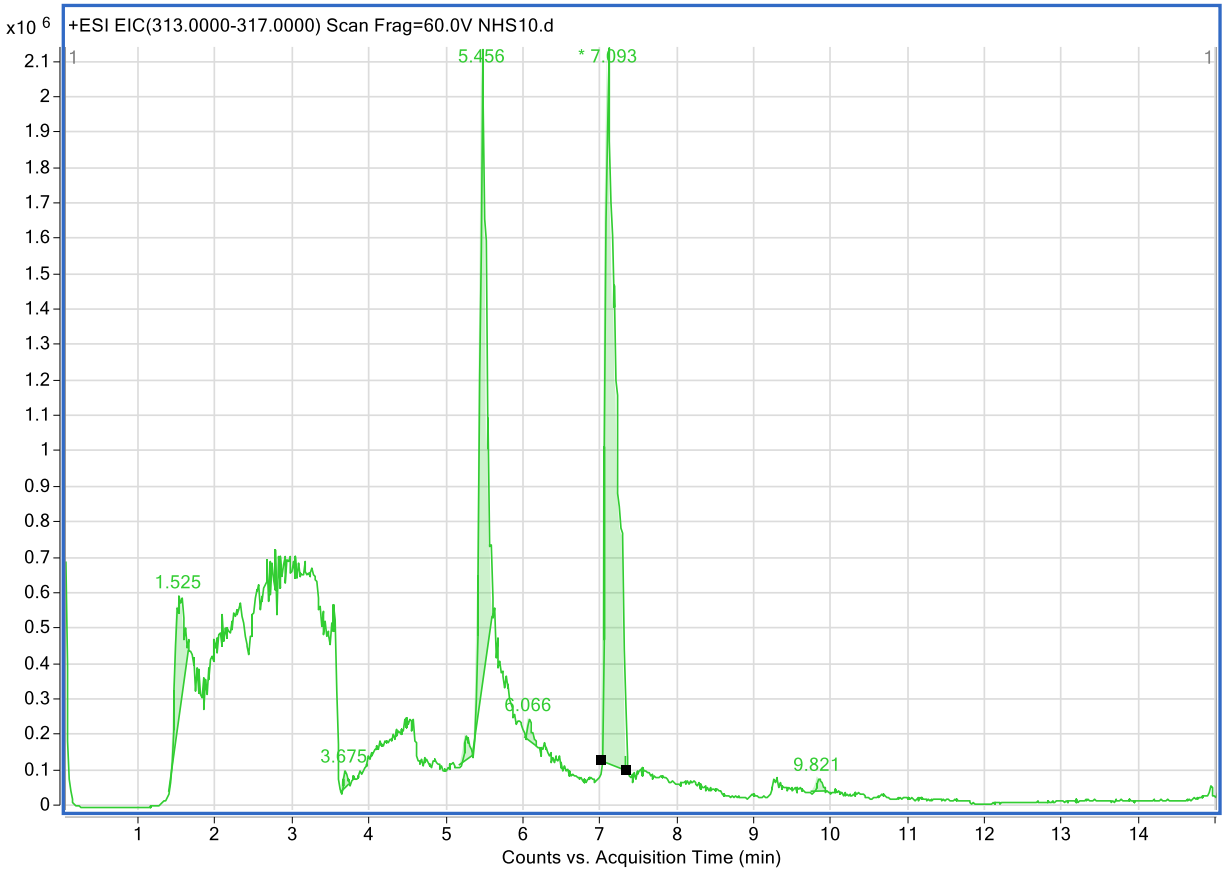
MC Run 2



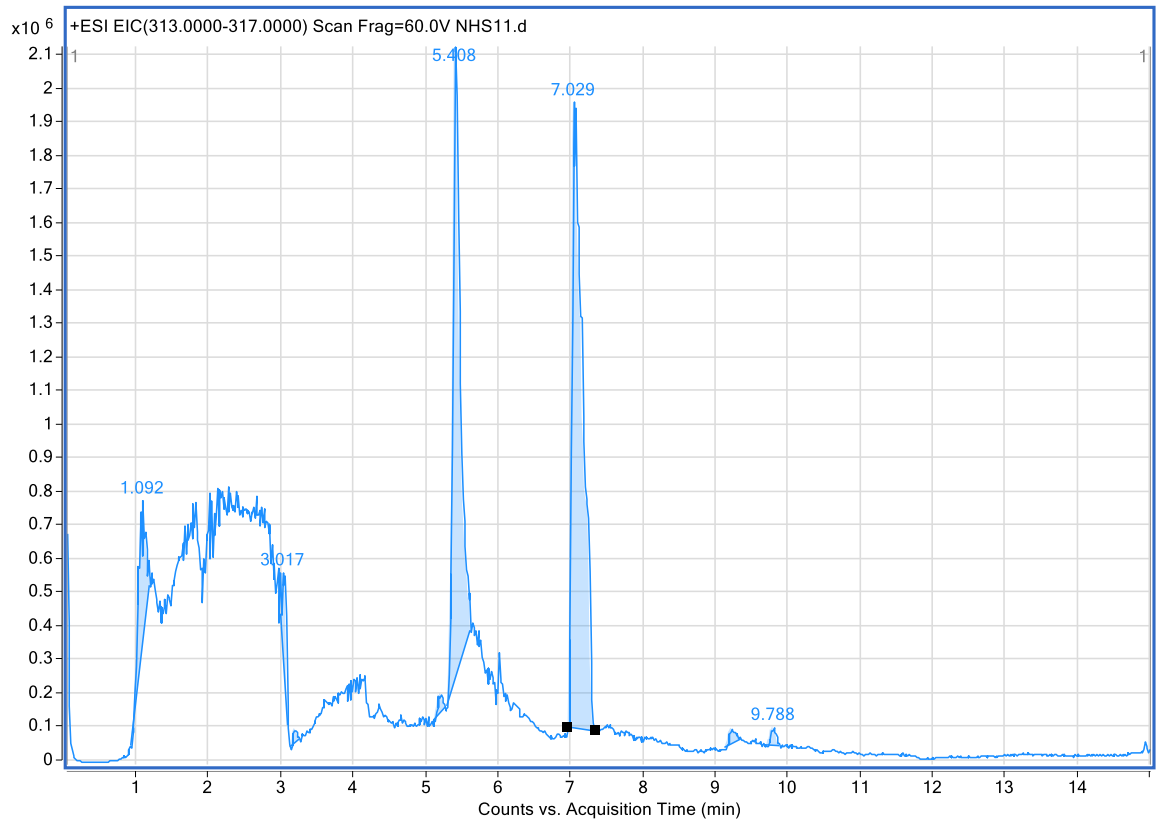
MC Run 3


+ESI EIC(313.0000-317.0000) Scan Frag=60.0V NHS9.d
x10 5
1.983
2.962
4.679
5.481
6.299
7.471
8.0
7.963
9.910
10.359
11.001
11.578
Counts vs. Acquisition Time (min)

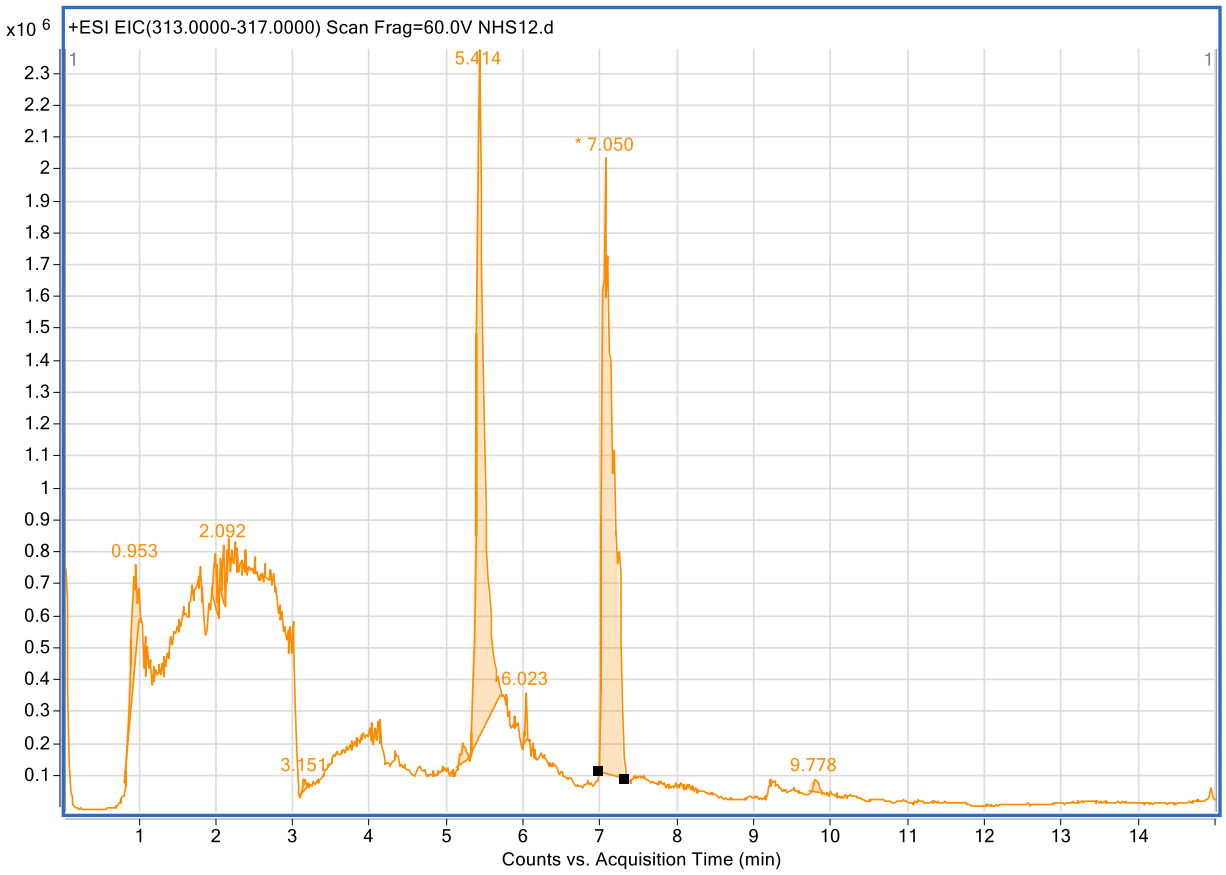
MA Run 1


x10 6
+ESI EIC(313.0000-317.0000) Scan Frag=60.0V NHS10.d
5.456
* 7.093
1.525
3.675
6.066
9.821
Counts vs. Acquisition Time (min)

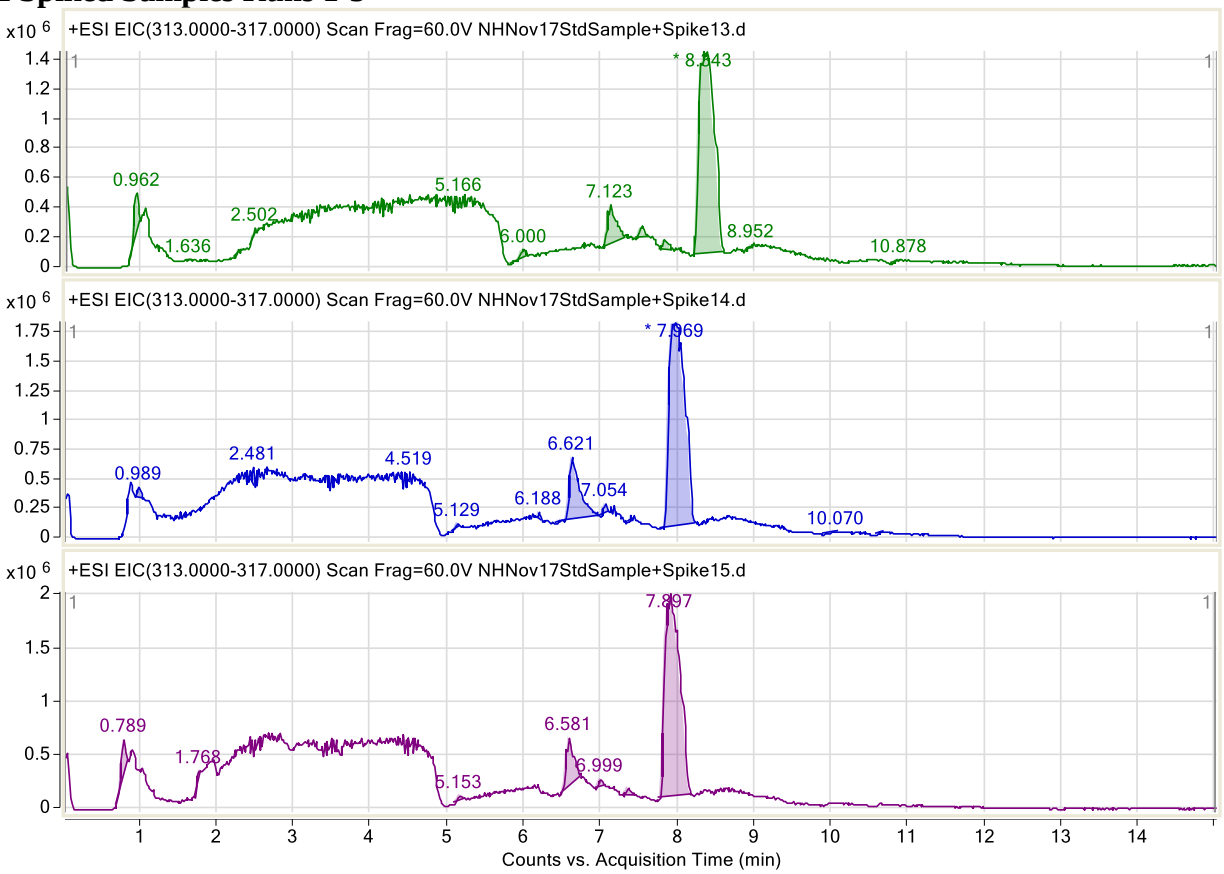
MA Run 2


x10 6
+ESI EIC(313.0000-317.0000) Scan Frag=60.0V NHS11.d
5.408
7.029
1.092
3.017
9.788
Counts vs. Acquisition Time (min)

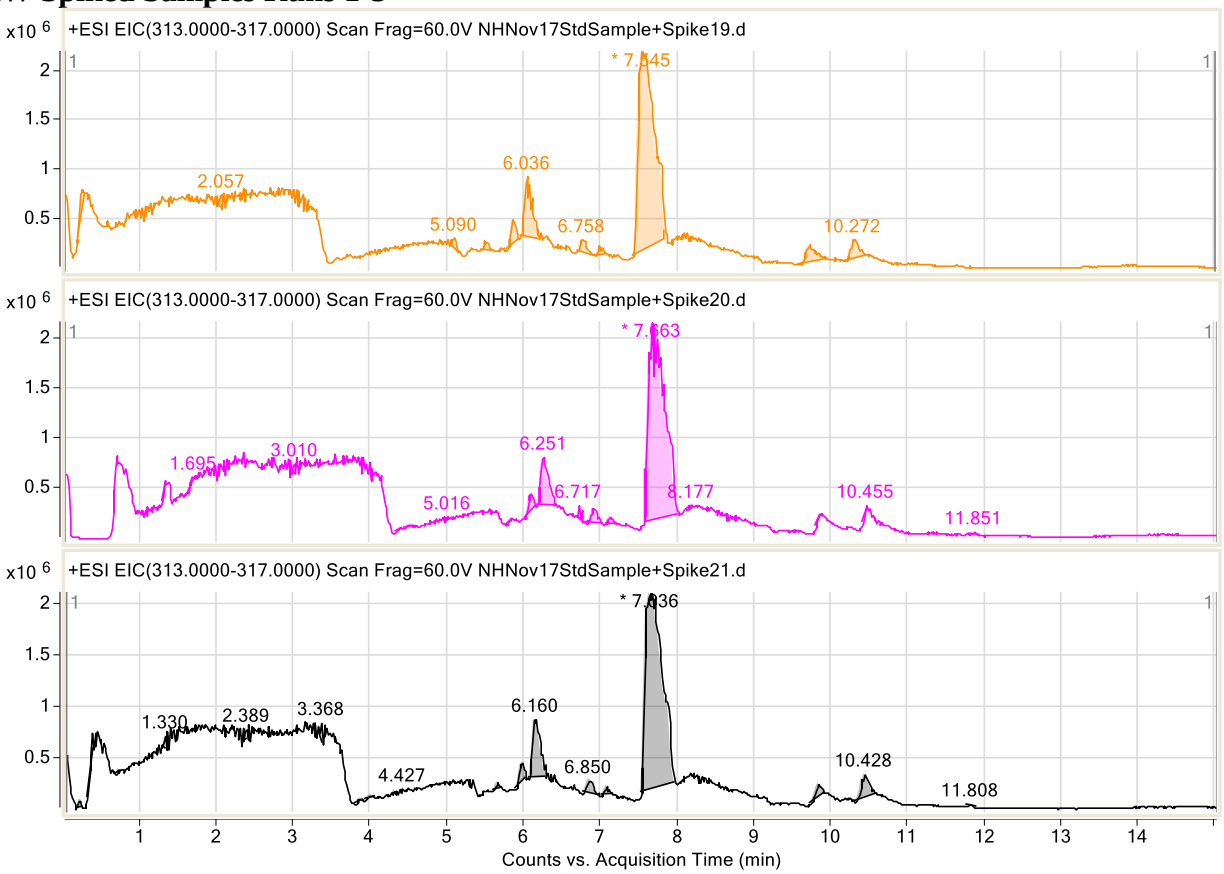
MA Run 3


x10 6
+ESI EIC(313.0000-317.0000) Scan Frag=60.0V NHS12.d
5.414
* 7.050
2.092
0.953
6.023
3.151
9.778
Counts vs. Acquisition Time (min)

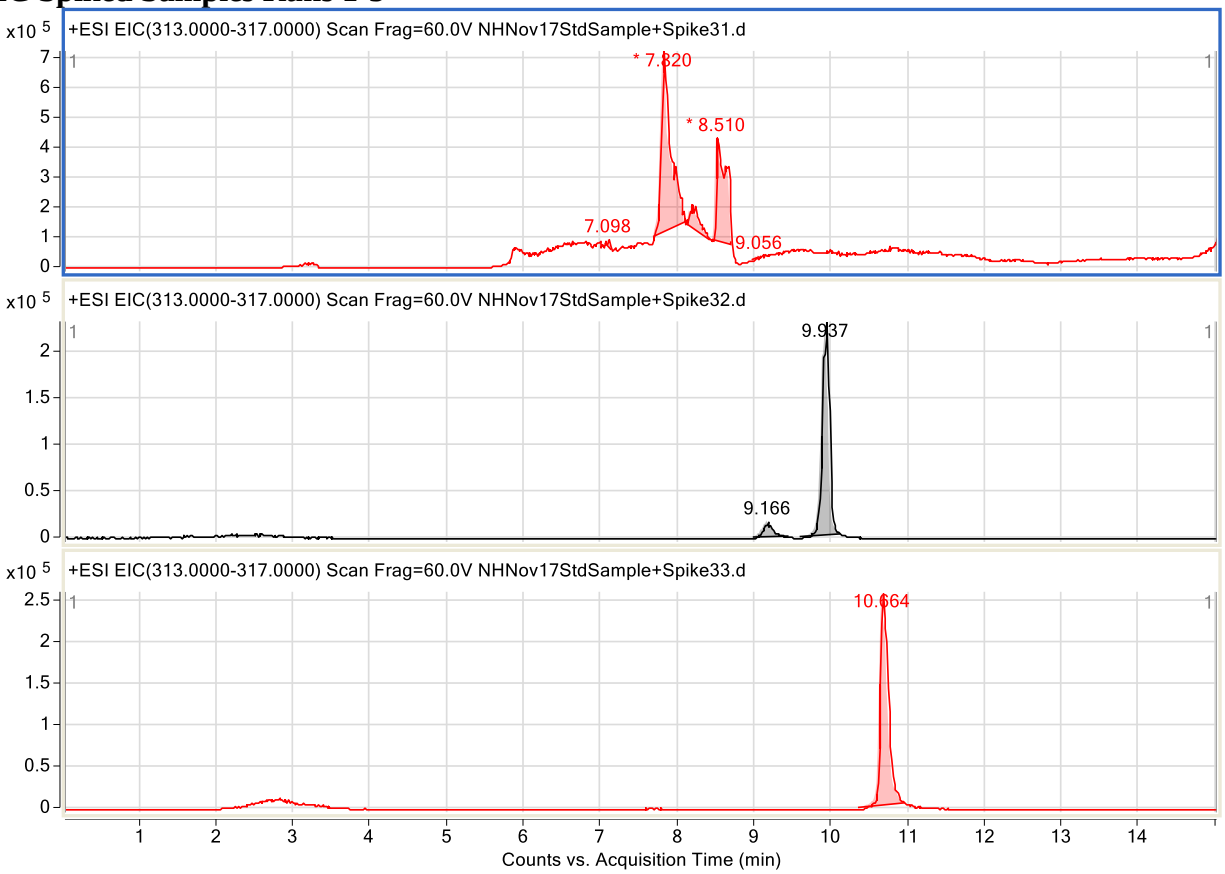
BI Spiked Samples Runs 1-3



RW Spiked Samples Runs 1-3



MC Spiked Samples Runs 1-3



MA Spiked Samples Runs 1-3