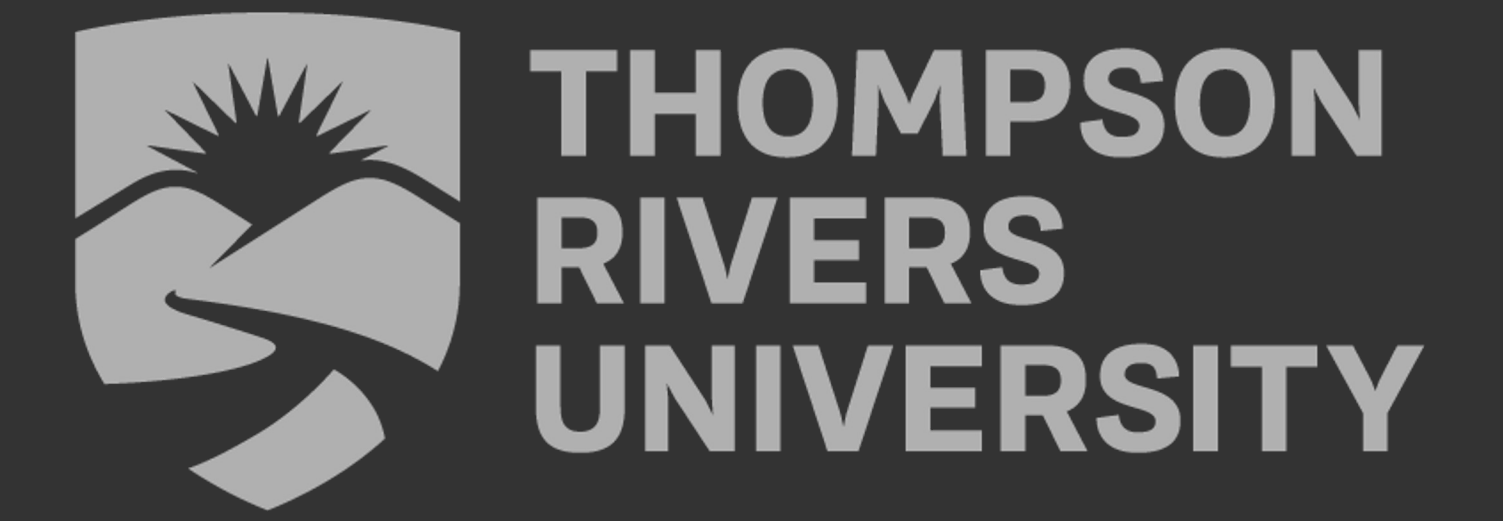# Determination of Cannabidiol in Various Cannabis Flower Buds using Liquid Chromatography – Mass Spectrometry

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Objective

Develop a sensitive and accurate method to detect the amount of cannabidiol (CBD) present in cannabis flower bud samples, using liquid chromatography – mass spectrometry (LC-MS).

Introduction

CBD is an active component in cannabis that is responsible for its calming properties, acting as an entourage to reduce psychoactive components commonly found in cannabis. Pharmacological benefits include evidence as an anti-epileptic agent, anti-inflammatory properties, anxiolytic effects, as well as potential applications for some dermatitis and related dermal treatment. Cannabis sample information is found in Table 1.
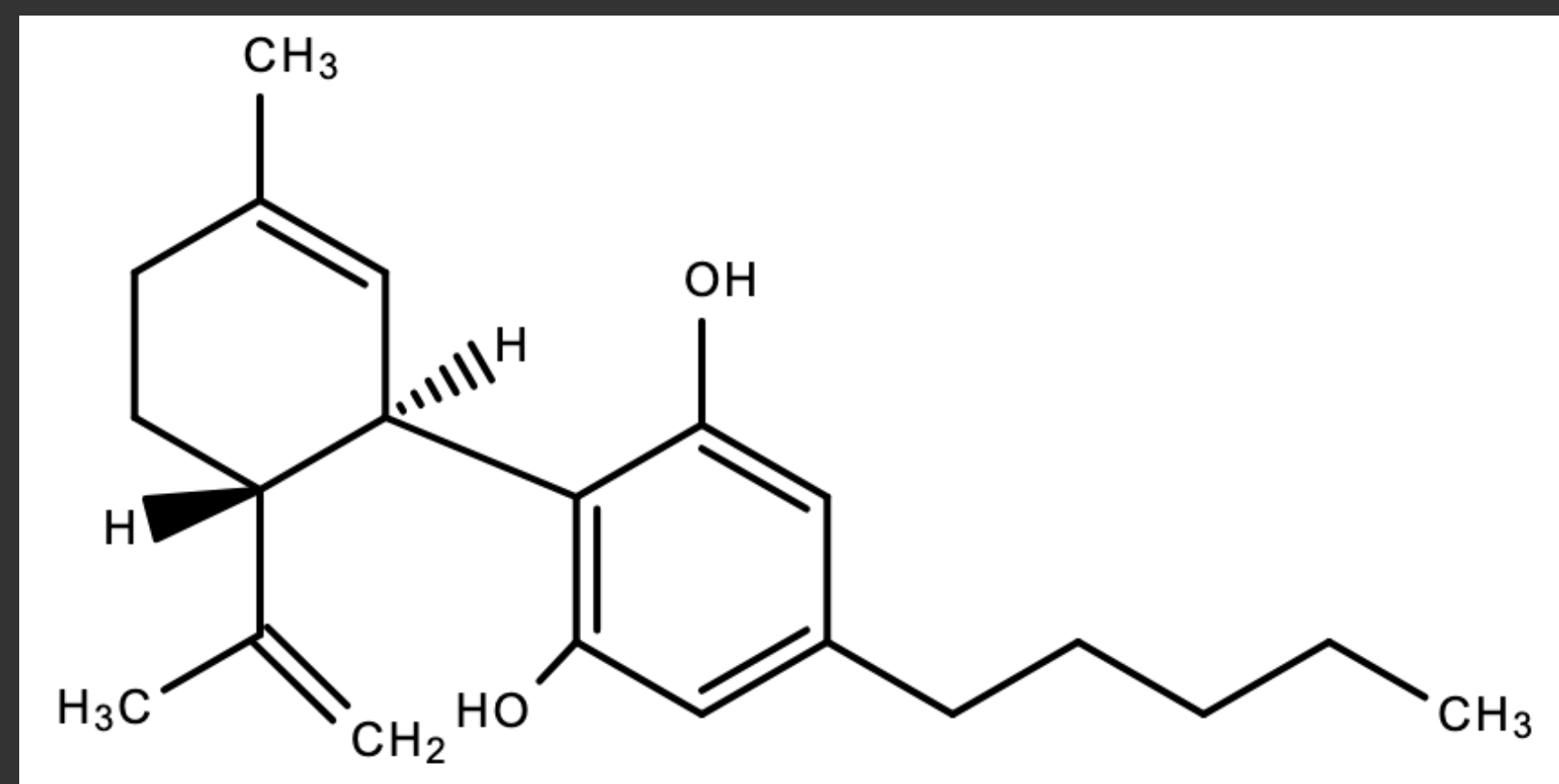

Figure 1. Structure of cannabidiol.

Table 1. Cannabis sample names and concentrations.

Sample	Labelled THC Concentration (mg/g)	Labelled CBD Concentration (mg/g)	Company
Blue Iguana	3.30	< 0.10	Weed Me
Wappa	15.0	0.00	Redecan
Mandarin Cookies	3.80	< 0.10	Weed Me
Miracle 15 x Alien Cookies	5.19	< 0.50	Holy Mountain

Figure 2. Cannabis samples. a) Blue Iguana[1] b) Wappa[4] c) Mandarin Cookies[3] d) Miracle 15 x Alien Cookies[2].

Standardization of testing is required to ensure accurate product labeling, especially with increased variability of results in testing facilities. Based on previous literature, LC-MS was chosen as it was found to be the most sensitive at detecting cannabis in multiple matrices[6].

Experimental

Method:
- CBD standards were prepared from a 50 ppm stock solution, ranging from 5.0 - 25.0 ppm.
- CBD was extracted from the cannabis samples by grinding and diluting in 25 mL methanol. Samples were filtered through a 0.45 µm Nylon filter, and pre-concentrated by nitrogen evaporation. Samples were reconstituted in 4.0 mL methanol.
- Samples were diluted with 18 MOhm water in LC-MS vials prior to analysis.
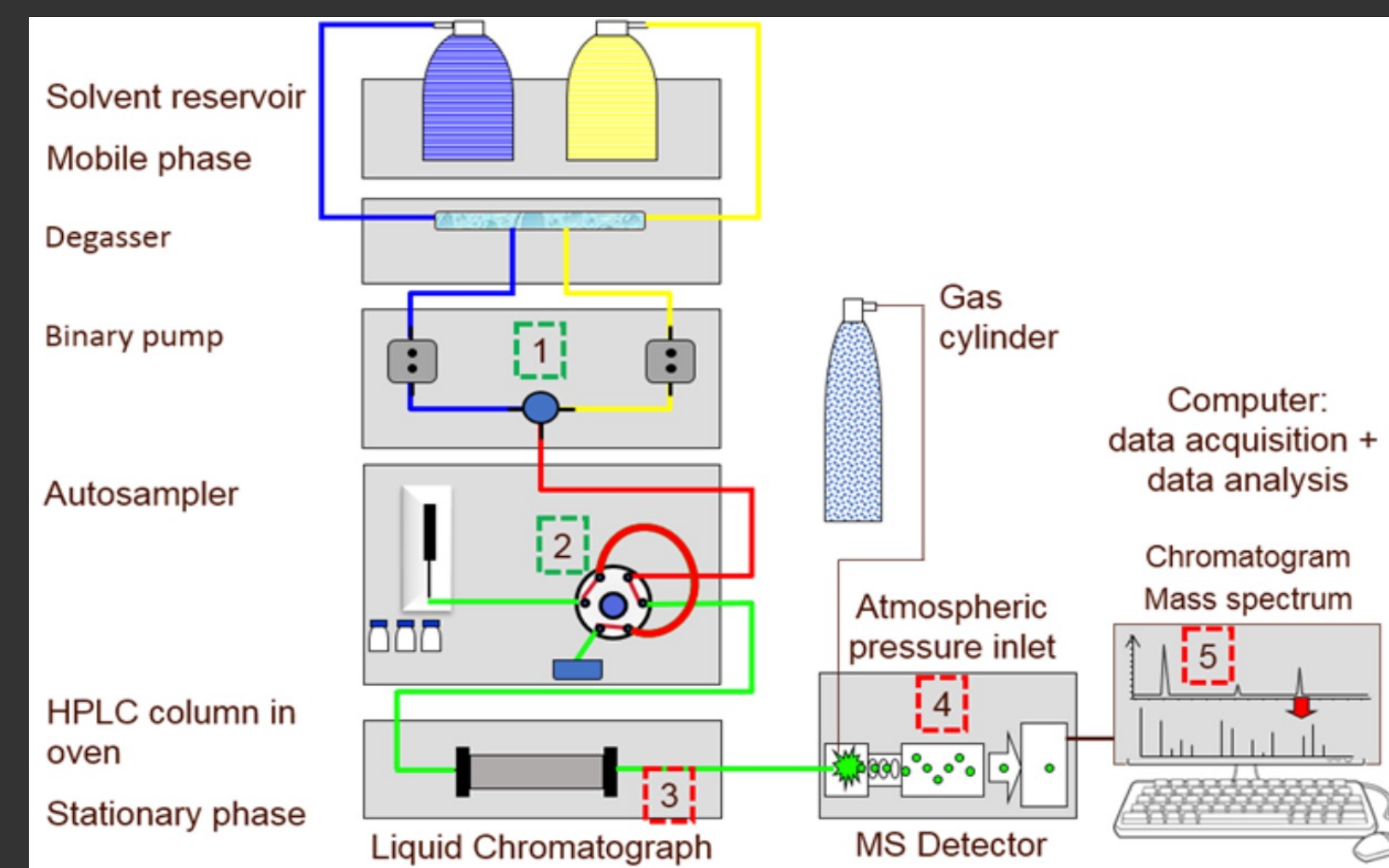
Instrument:

Figure 3. Schematic of the LC-MS[5].

Figure 4. Agilent 1200 LC-6530 QTOF Mass Spectrometer.

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

|	
Column	2.1 x 100 mm
Internal Diameter	1.8 µm
Ionization Source	ESI+
VCap	3000 V
Fragmentor Voltage	60.0 V
Gas Temperature	350°C
Drying Gas	8.0 L/min
Nebulizer	15 psig
Sheath Gas Temperature	325 °C
Sheath Gas Flow	8.0 L/min
Acquisition	100 – 500 m/z
Flow Rate	0.5 mL/min
Injection Size	5.0 µL
Column Temperature	30°C
Solvents	A: 25% methanol and 0.3% formic acid in water B: 0.3% formic acid in acetonitrile

Results
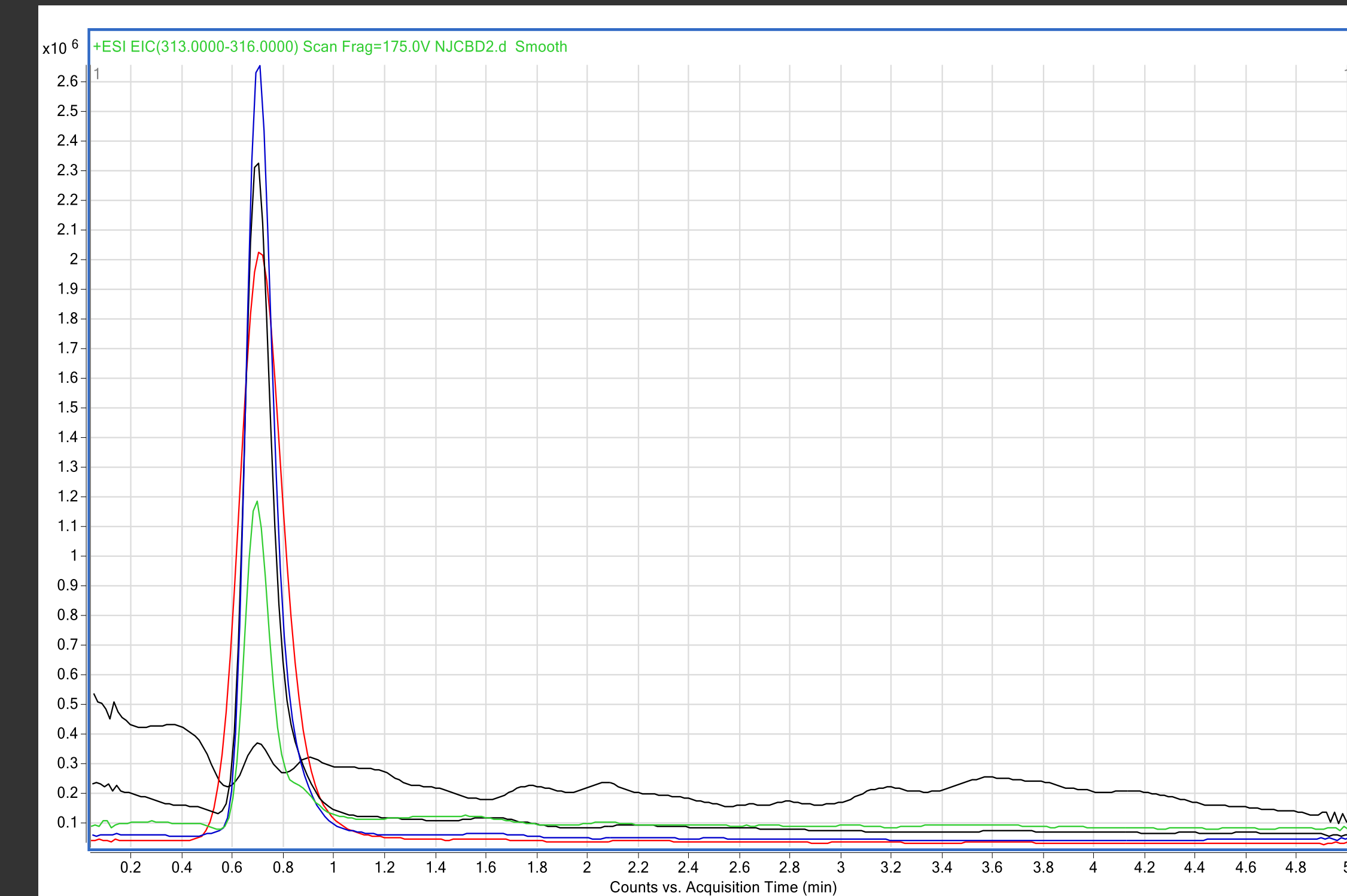
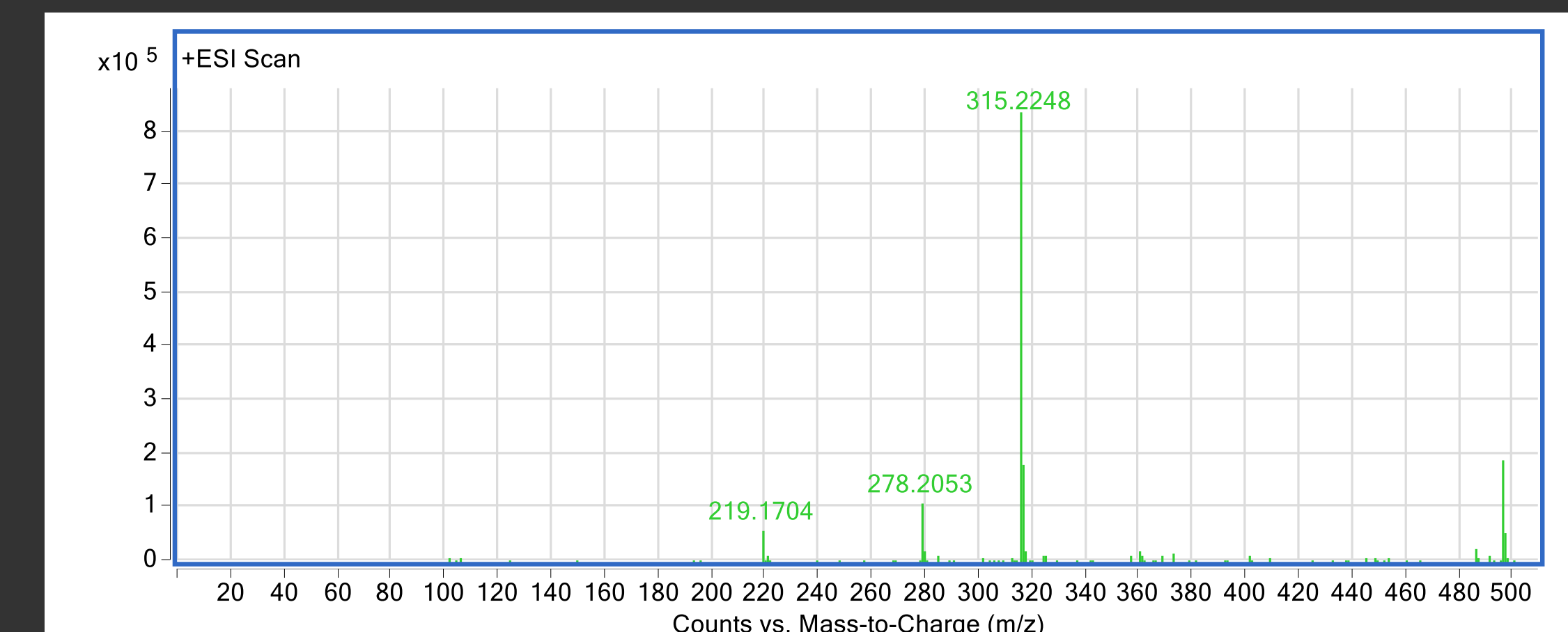Figure 5. Chromatograms of CBD standards.

Figure 6. Mass spectra of the 10 ppm standard.

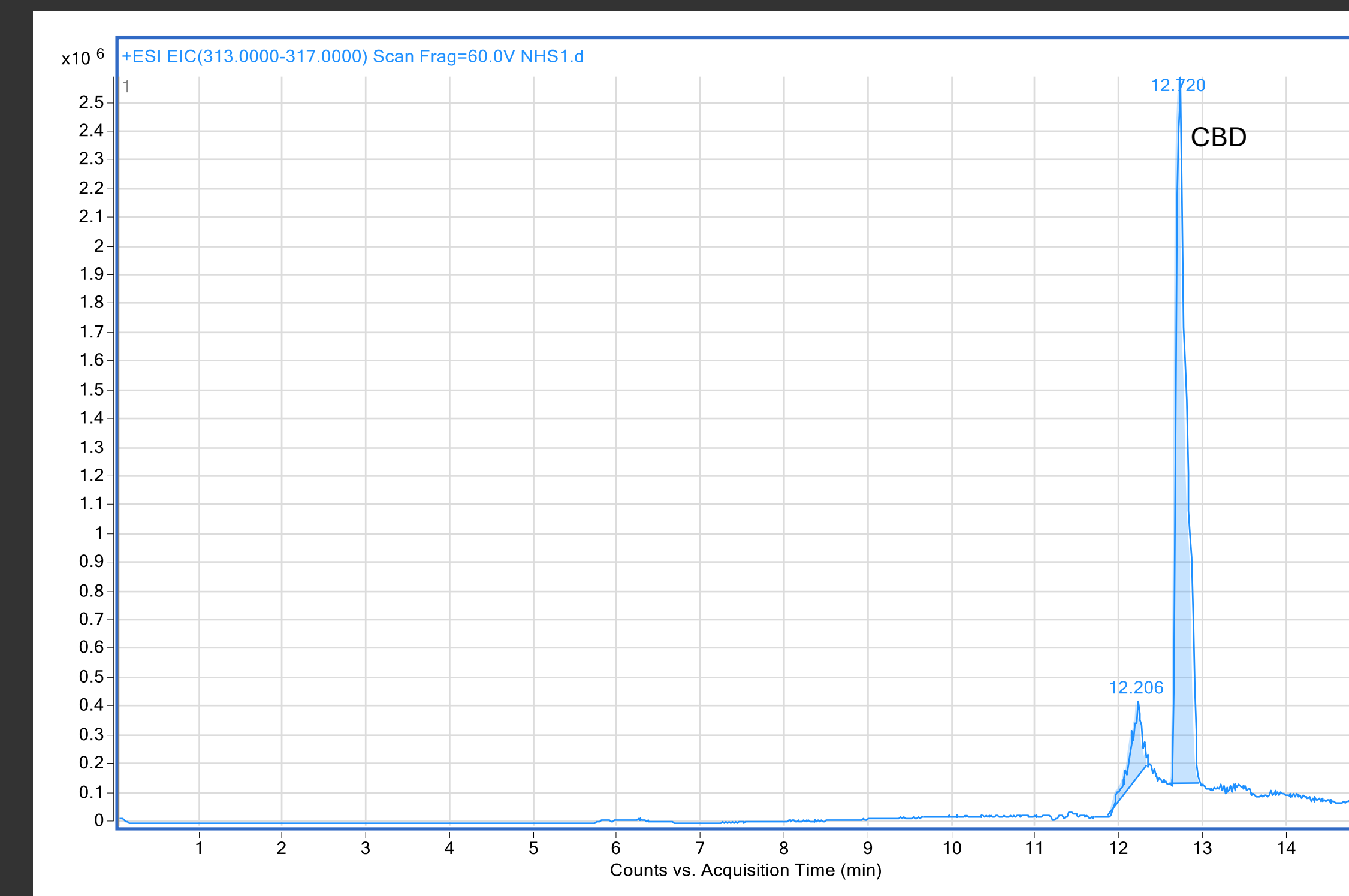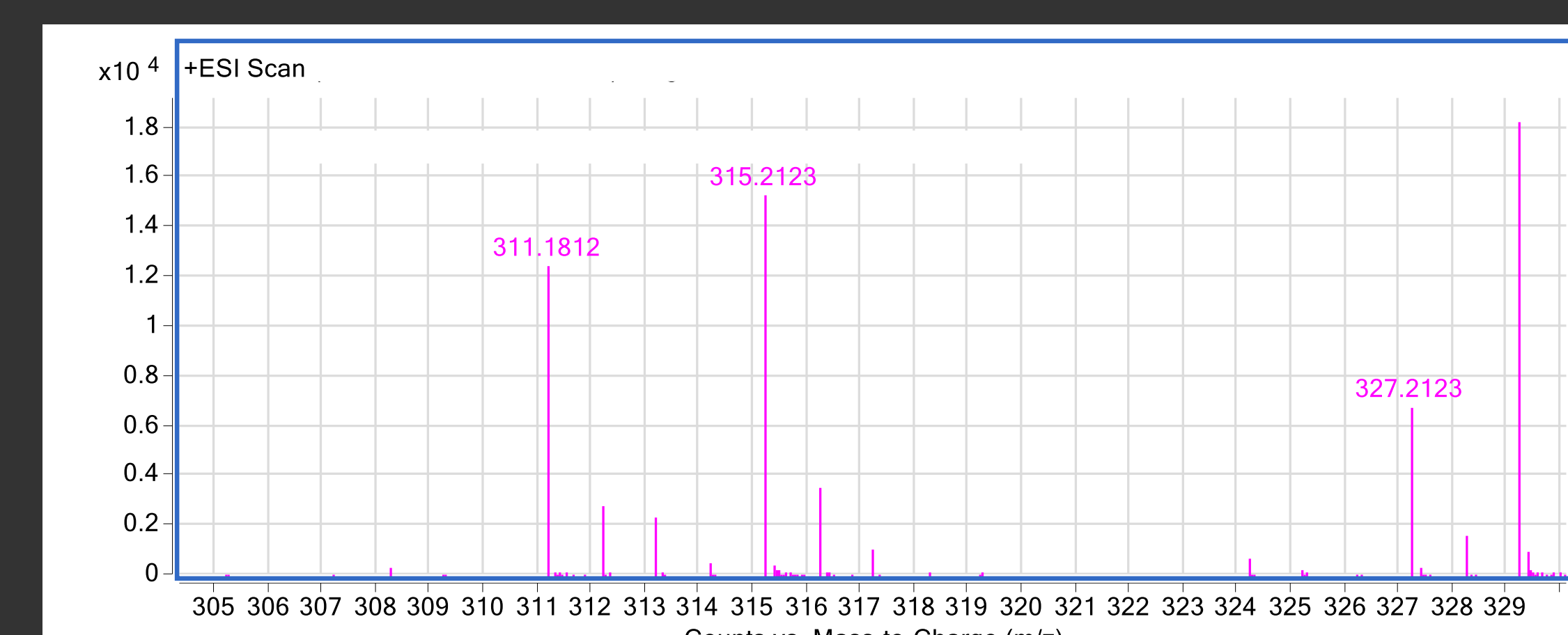Figure 7. Chromatogram of CBD in Blue Iguana flower bud samples.

Figure 8. Mass spectra for the cannabis flower bud sample.

Results

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

Samples	CBD Concentration in Samples (ppm)	Concentration in (mg/g)	Labelled CBD Concentration (mg/g)
Blue Iguana (BI)	21.565	0.345	< 0.100
Wappa (RW)	22.193	0.355	0.000
Mandarin Cookies (MC)	1.302	0.010	< 0.100
Miracle 15 x Alien Cookies (MA)	11.761	0.094	< 0.500

Conclusion

- Chromatogram results show increasing concentration of CBD
- CBD was detected in higher values than labelled in 2 of the 4 samples
- 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).

Future work

- Validate the method by comparing concentrations to values obtained using a capillary electrophoresis method to further improve results.
- Determine limit of detection (LOD) and limit of quantification (LOQ) to ensure sensitivity of the method.
- Work will be extended to other parts of the cannabis plant, and extend the study to flower buds from homegrown cannabis plants.

Acknowledgements

References

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3. Anonymous, (n.d.). Mandarin Cookie Sativa Dominant Dry Flower. 1g, 3.5g. *Weed Me.* https://weedme.ca/product/mandarin-cookie/
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6. McRae, G., Melanson, J.E., (2020). Quantitative determination and validation of 17 cannabinoids in cannabis and hemp using liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem* 412, 7381–7393. https://doi.org/10.1007/s00216-020-02862-8