

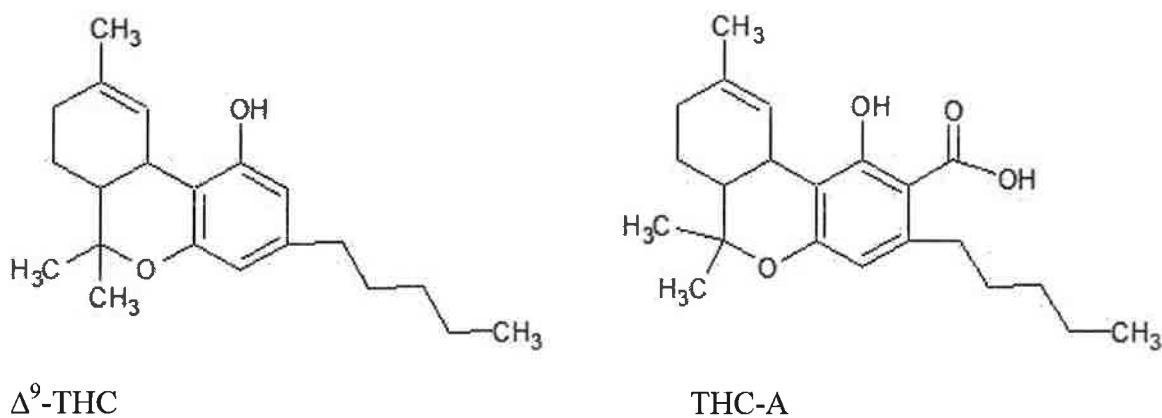
IV. Separation:

When quantitating chemical compounds it is important to know that the measured amount is limited to the analyte of interest. In the case of gas chromatography/mass spectroscopy this means the gas chromatograph method should separate as completely as possible various components that might be present in the sample. For THC analysis, it would be necessary to separate the different cannabinoids.

Developing a GC/MS method to separate compounds can be a difficult process. Luckily, our laboratory had developed a method for qualitatively analyzing a multitude of compounds including THC. This method was altered in order to shorten the run time and to improve separation of the cannabinoids in the plant material. The temperature portion of the method was the only parameter that was altered. It was changed from a ten minute method where the temperature was ramped from 100°C to 280°C at 70°C/minute to a five minute method where the temperature is held at 230°C for three and half minutes then ramped to 280°C at 50°C/minute. This method resulted in good separation between THC and cannabidiol (CBD) the predominant cannabinoid in industrial hemp samples.

It is known that tetrahydrocannabinolic acid (THC-A) breaks down into Δ^9 -tetrahydrocannabinol (THC) in UV light and at high temperatures (see figure 1). This occurs through a decarboxylation process. The injection ports of our gas chromatographs are at high temperature (250°C). We hypothesized that any THC-A present in a plant material sample would break down into THC when injected on our gas chromatograph instruments.

Figure 1:



This breakdown is unavoidable in a GC/MS quantitation method and it is necessary to understand that this methodology would only be capable of quantitating “total THC” (both THC and THC-A). Different instrumentation such as a liquid chromatograph triple quadrupole mass spectrometer or a high performance liquid chromatography method would be needed to distinguish between THC and THC-A. A Cerilliant standard of Δ^9 -Tetrahydrocannabinolic acid

(THC-A) was run on our instrumentation and a single peak confirmed by mass spectrometry to be THC was observed. This result confirmed the hypothesis about the thermal breakdown.

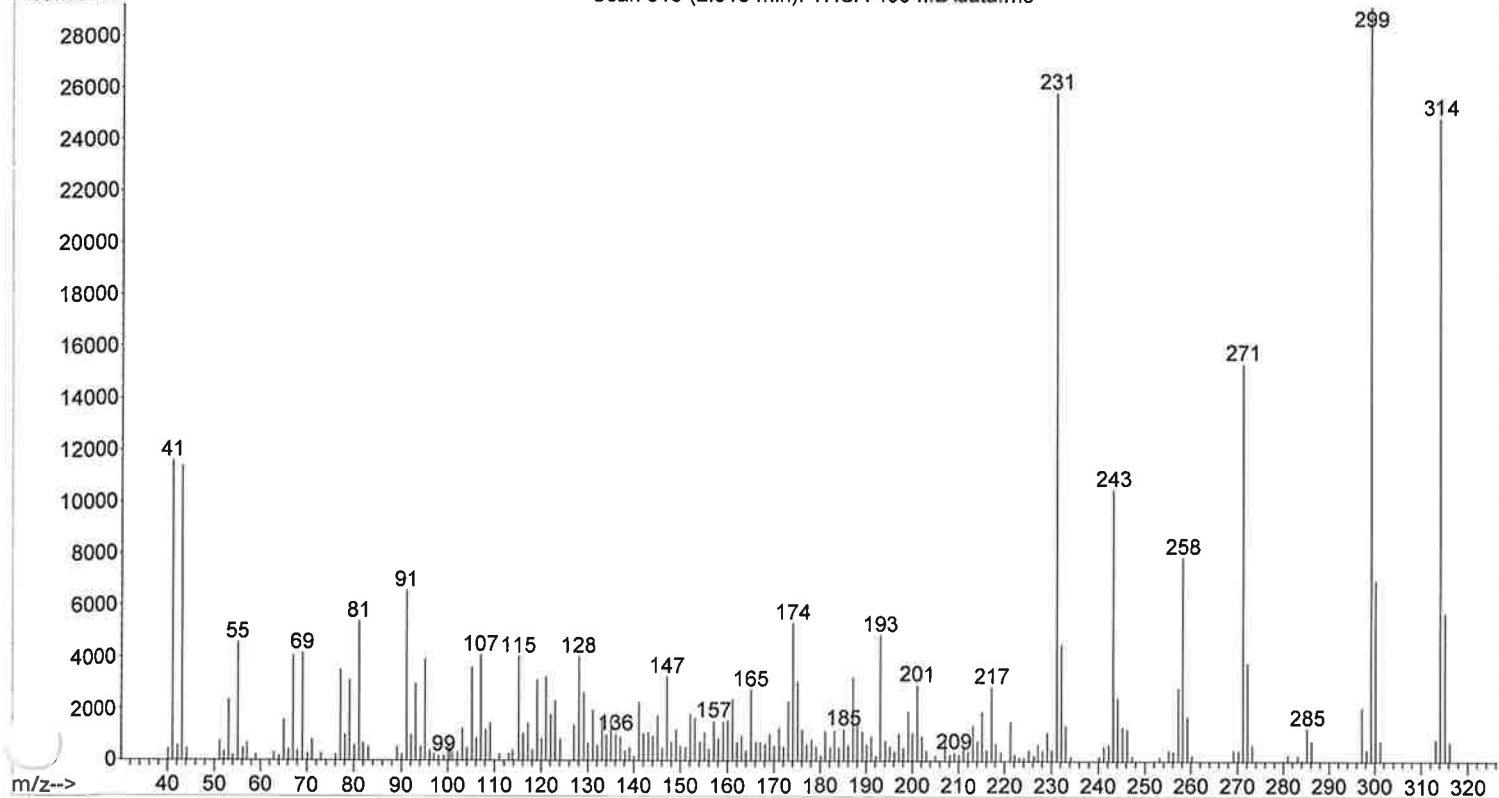
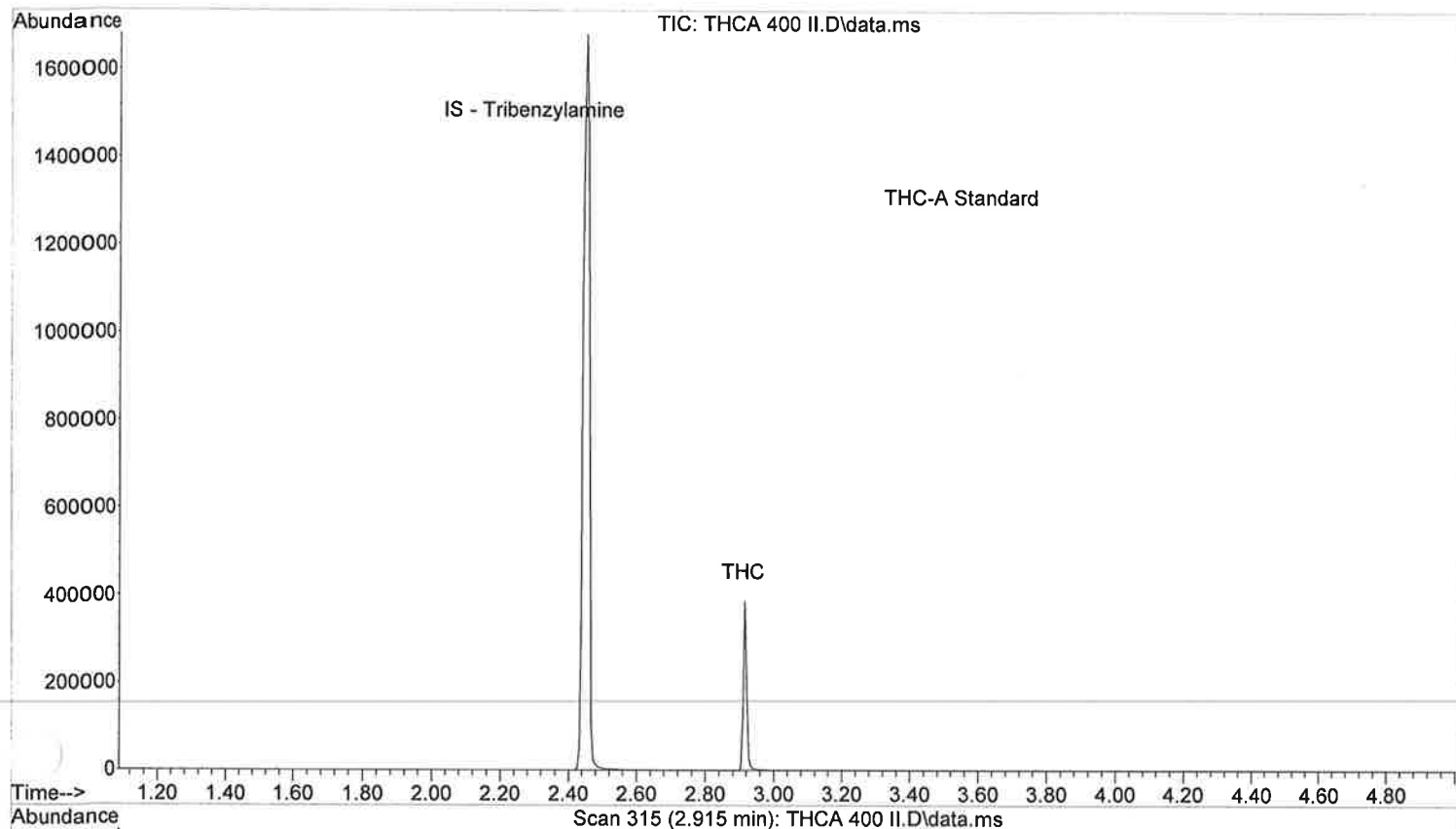
On 2/13/2019 multiple cannabinoid standards were run on a GC/MS (Heisenberg) using the THC-MS method. This experiment was designed to demonstrate the method's separation of different cannabinoids. The standards run were cannabigerol, cannabinol, Δ^8 - tetrahydrocannabinol, Δ^9 - tetrahydrocannabinol, cannabichromene, cannabidiol, and a mixture solution (cannabidiol, tetrahydrocannabinol, and cannabinol). These standards were all in a concentration of 1 mg/mL. The combined mixture in the study contained all the cannabinoid standards and the internal standard (Tribenzylamine) in concentrations of 1 mg/mL or 1000 μ g/mL.

Separation Study 2/13/2019

Heisenberg		Gumbo	
Compound	Retention Time	Compound	Retention Time
Cannabidiol	2.879	Cannabidiol	2.639
Cannabichromene	2.928	Cannabichromene	2.681
Δ^8 THC	3.444	Δ^8 THC	3.158
Δ^9 THC	3.622	Δ^9 THC	3.331
Cannabigerol	3.975	Cannabigerol	3.769
Cannabinol	4.03	Cannabinol	3.827
Cannabidiol (Mix 3)	2.879	Cannabidiol (Mix 3)	2.638
Δ^9 THC (Mix 3)	3.623	Δ^9 THC (Mix 3)	3.337
Cannabinol (Mix 3)	4.028	Cannabinol (Mix 3)	3.83
Combined Mixture		Combined Mixture	
Tribenzylamine	2.165	Tribenzylamine	1.97
Cannabidiol	2.862	Cannabidiol	2.605
Cannabichromene	2.913	Cannabichromene	2.651
Δ^8 THC	3.418	Δ^8 THC	3.112
Δ^9 THC	3.599	Δ^9 THC	3.28
Cannabigerol	3.967	Cannabigerol	3.736
Cannabinol	4.014	Cannabinol	3.796

This method separates all the standards used in this study and would be appropriate for samples from case work that might have multiple cannabinoids present.

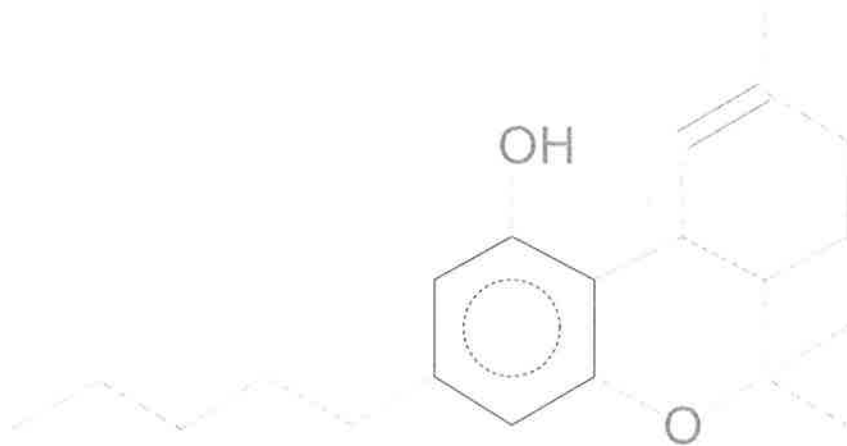
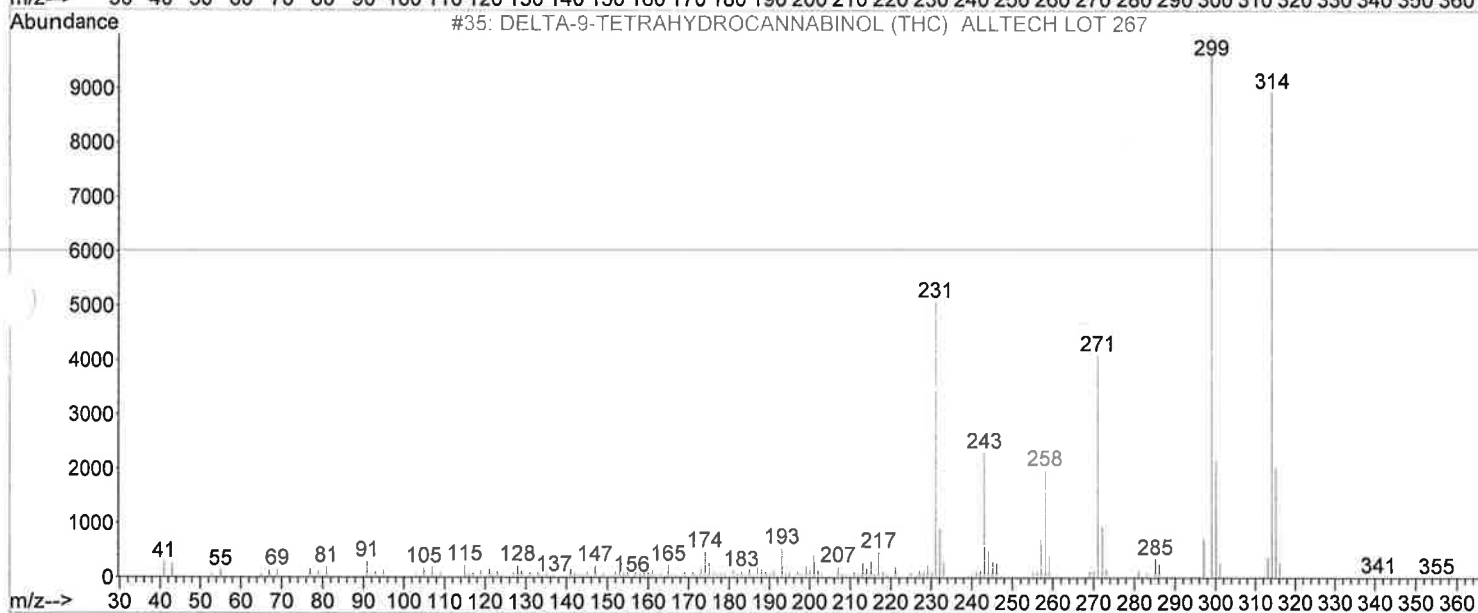
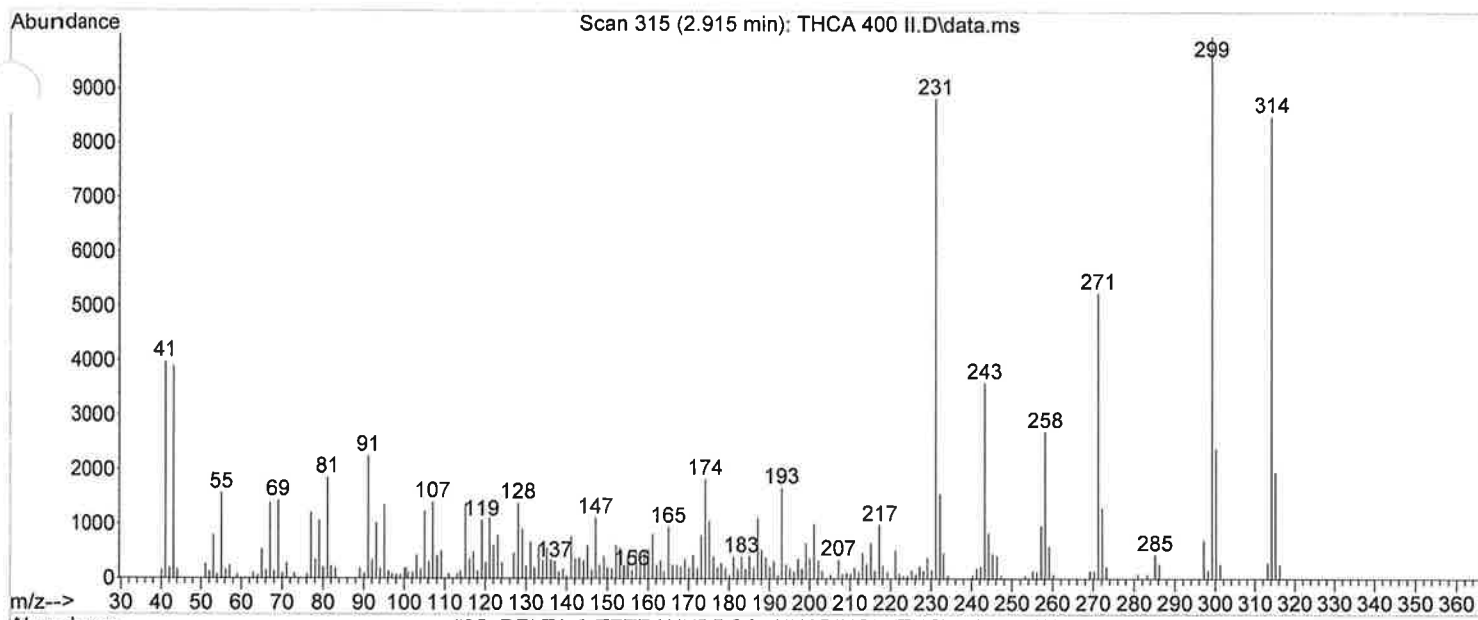
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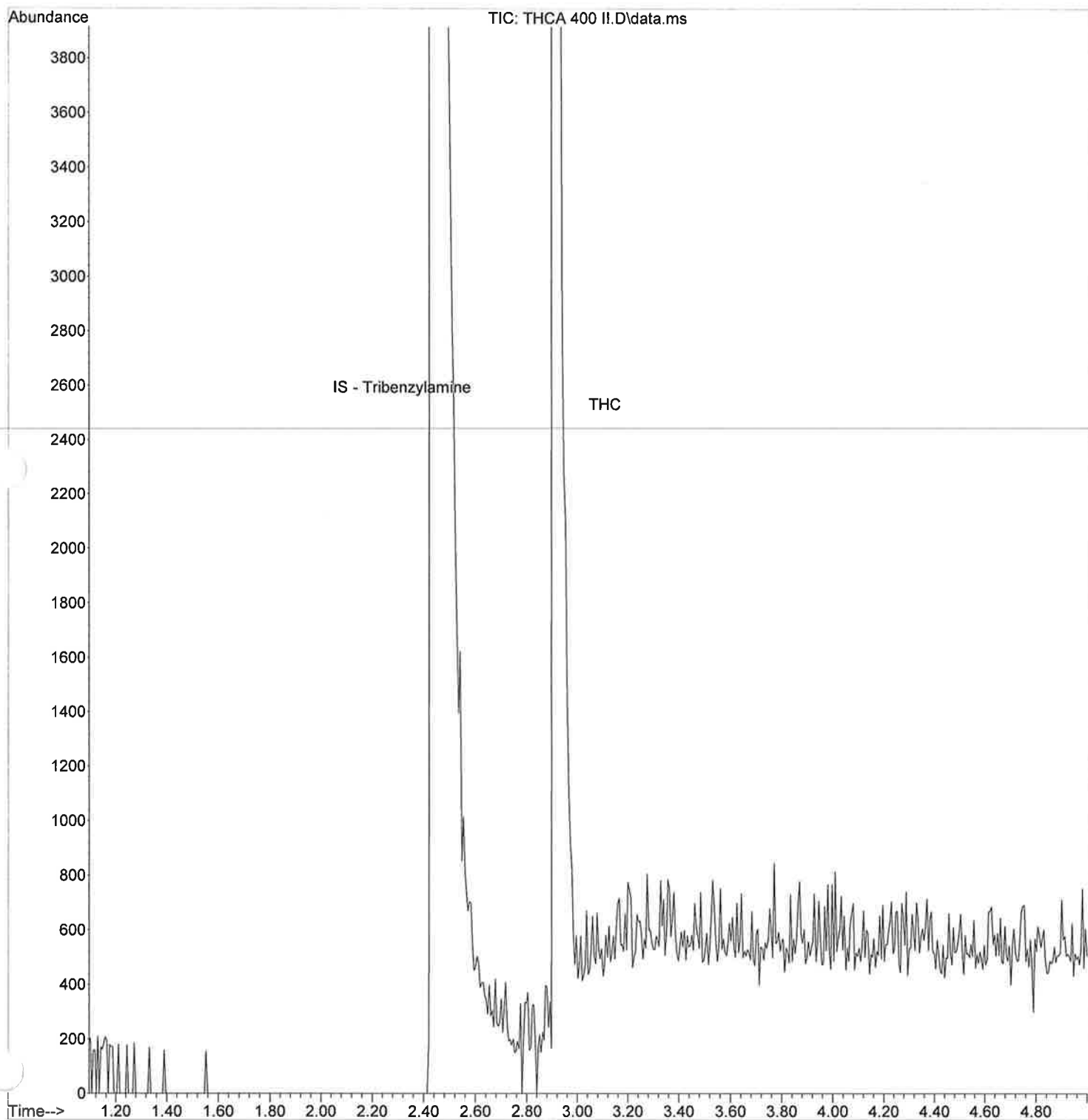
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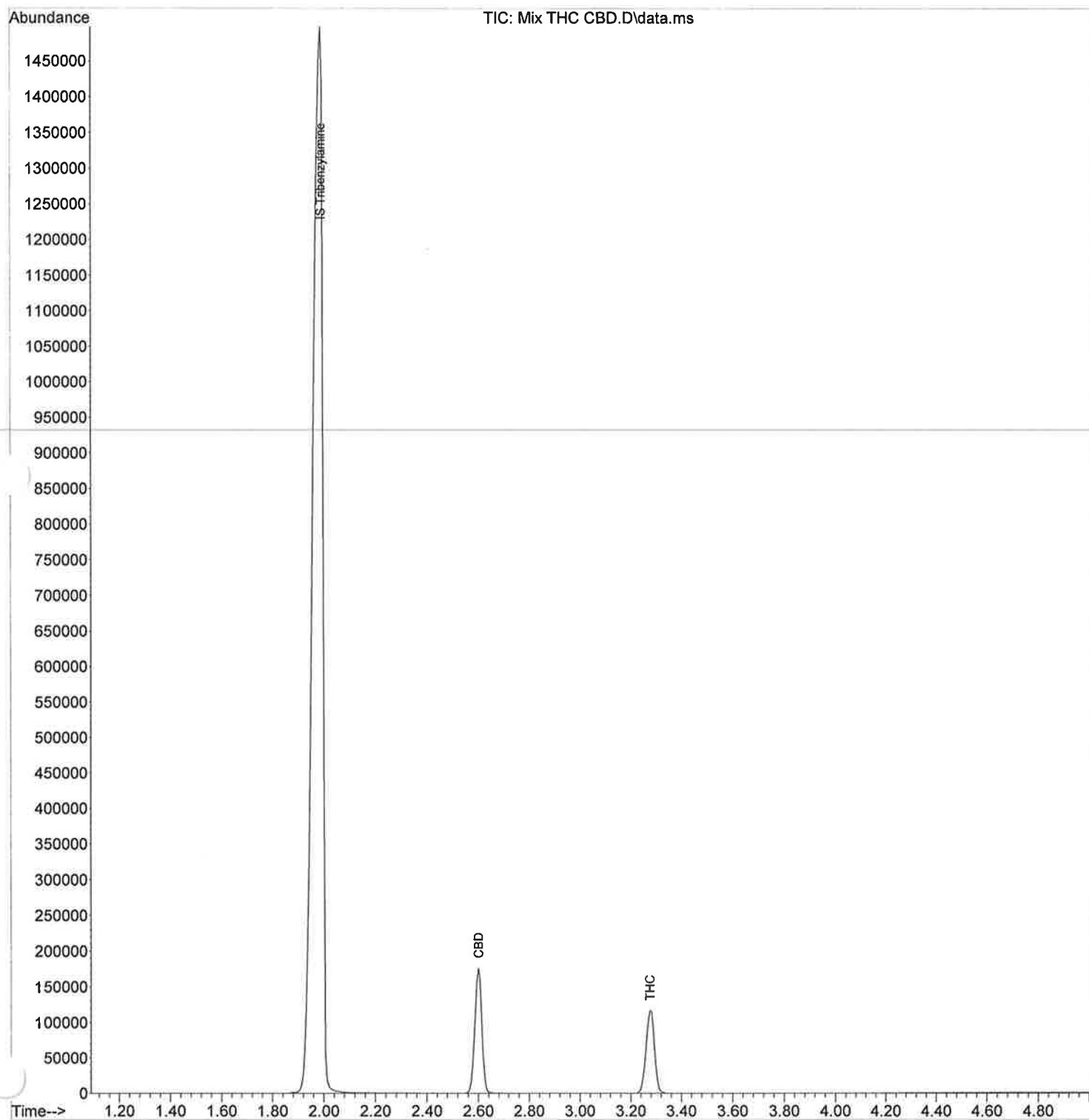
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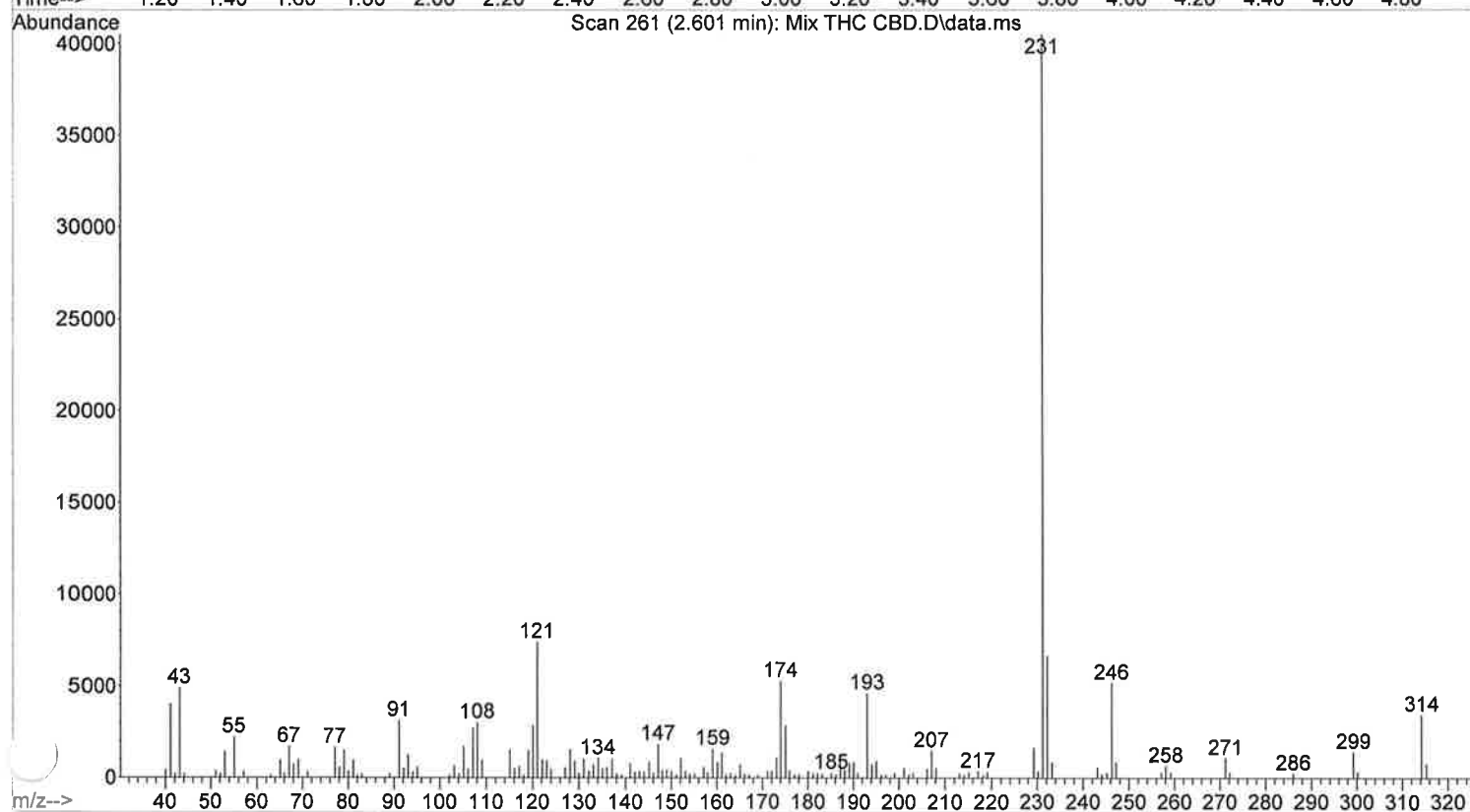
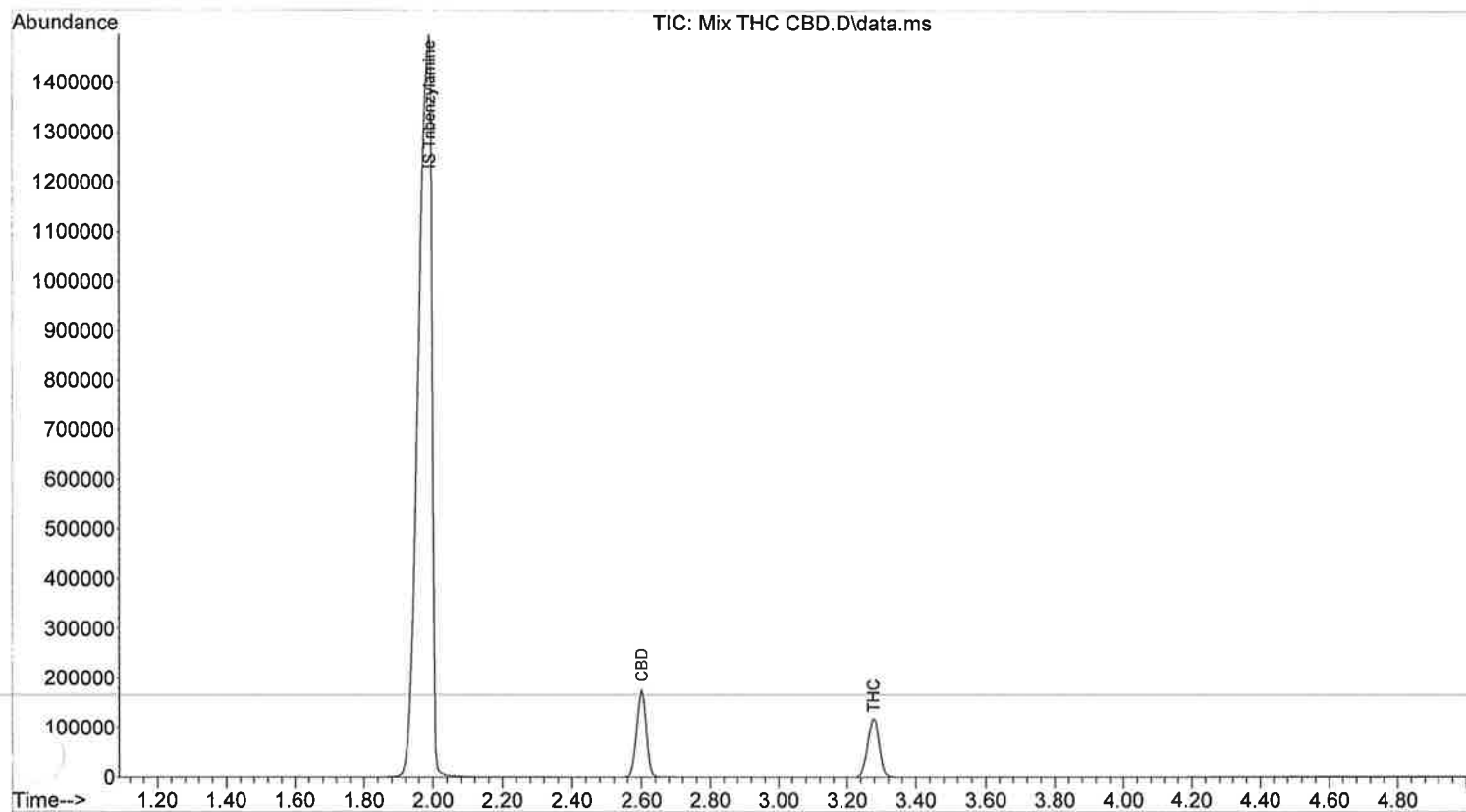
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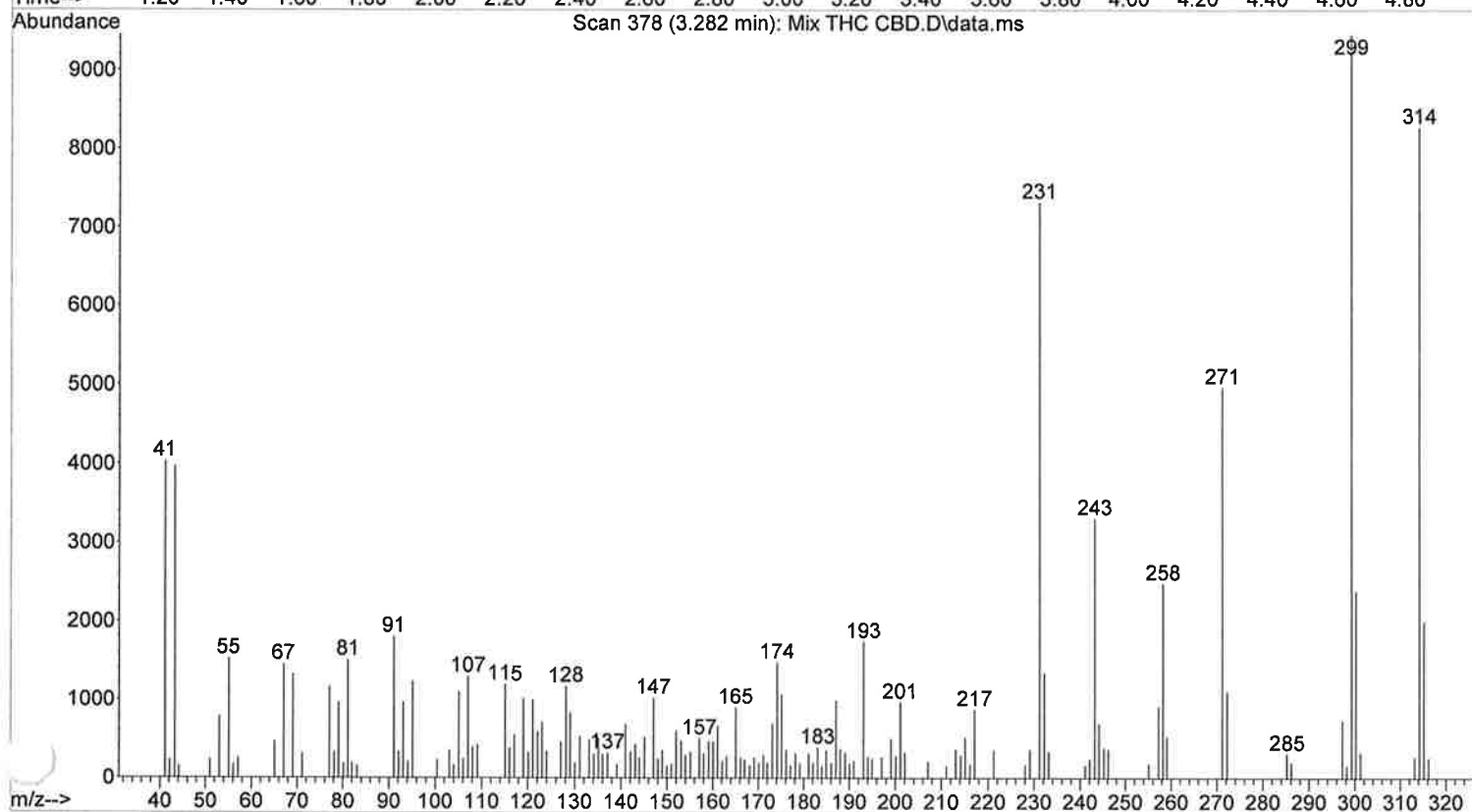
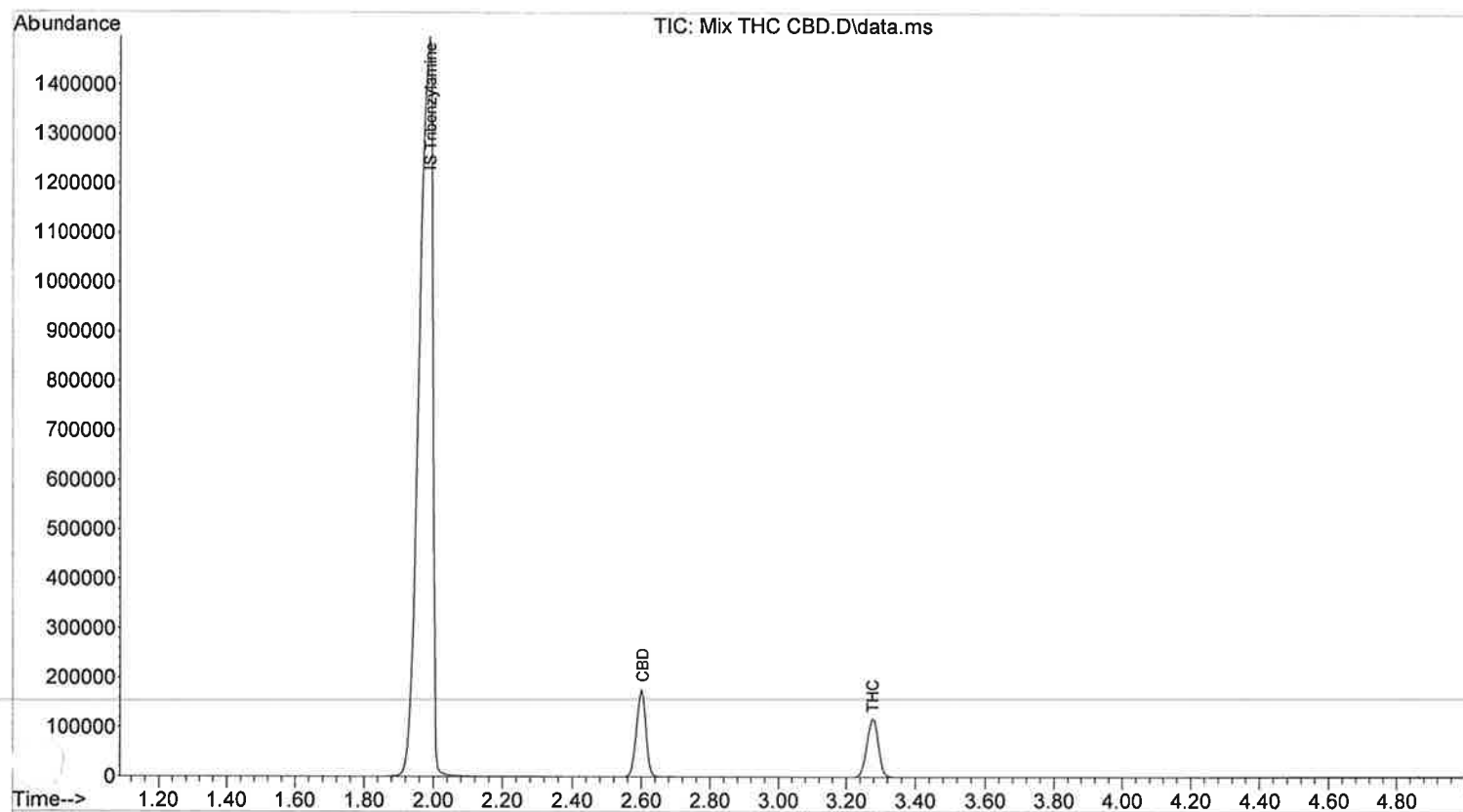
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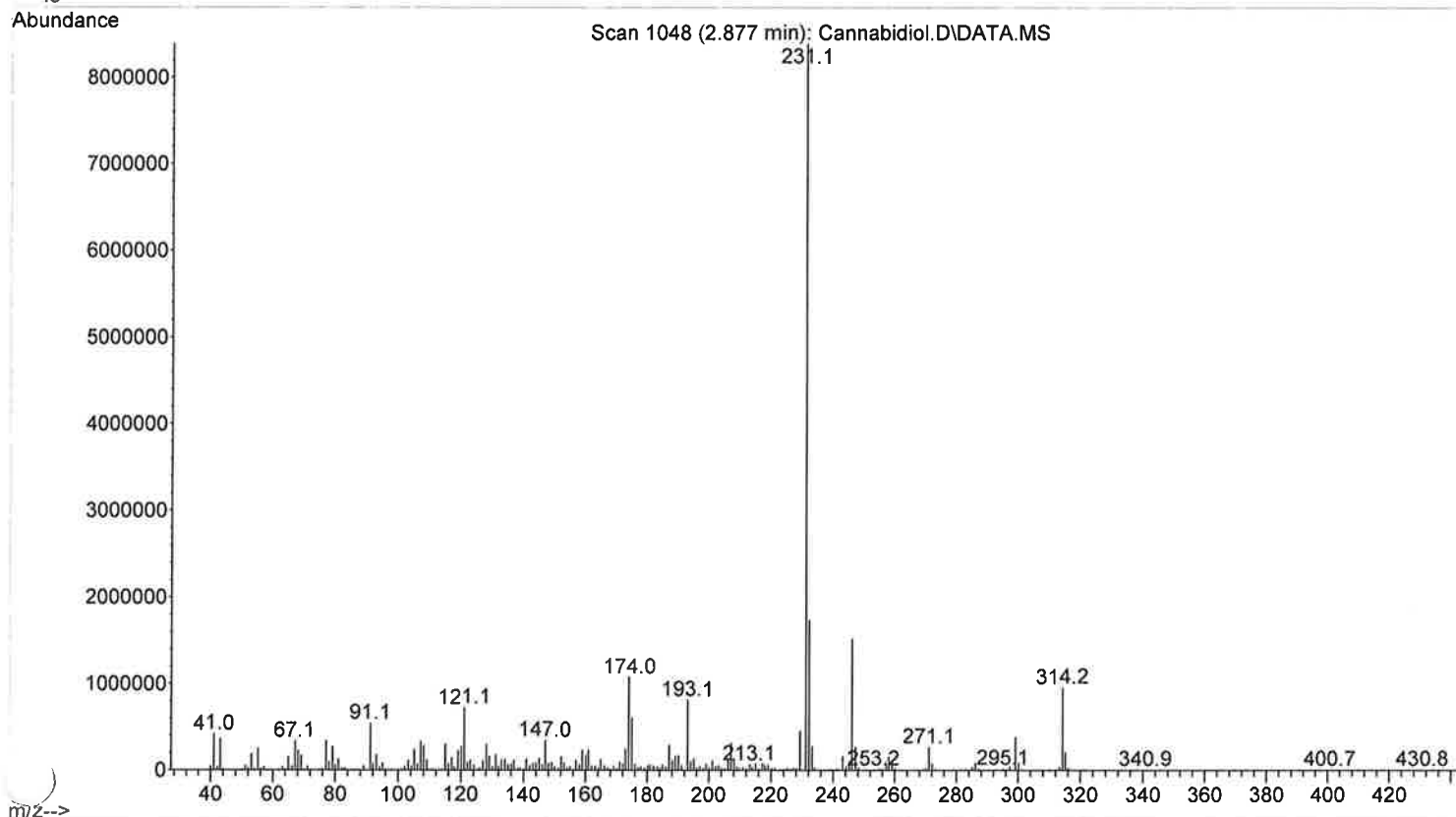
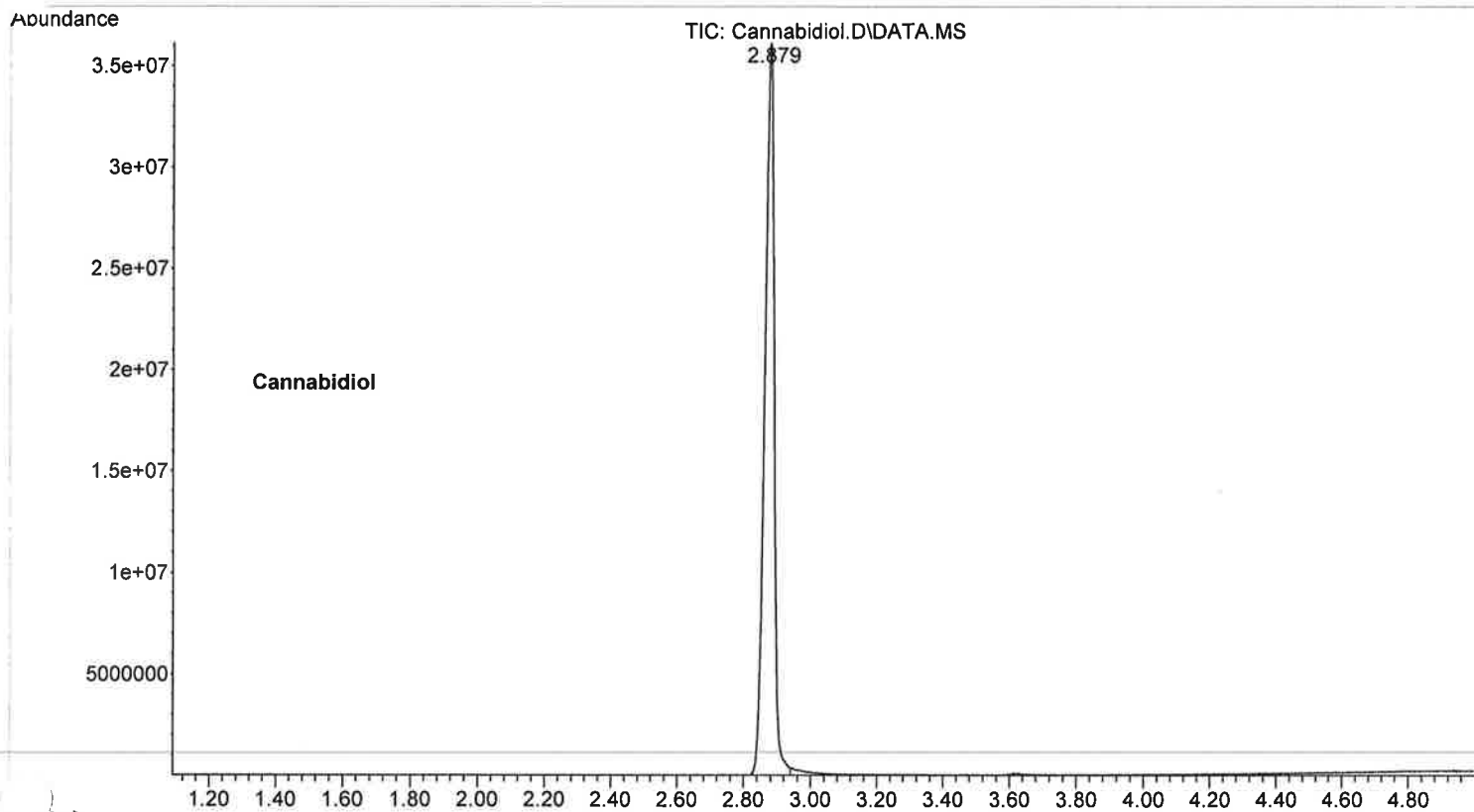
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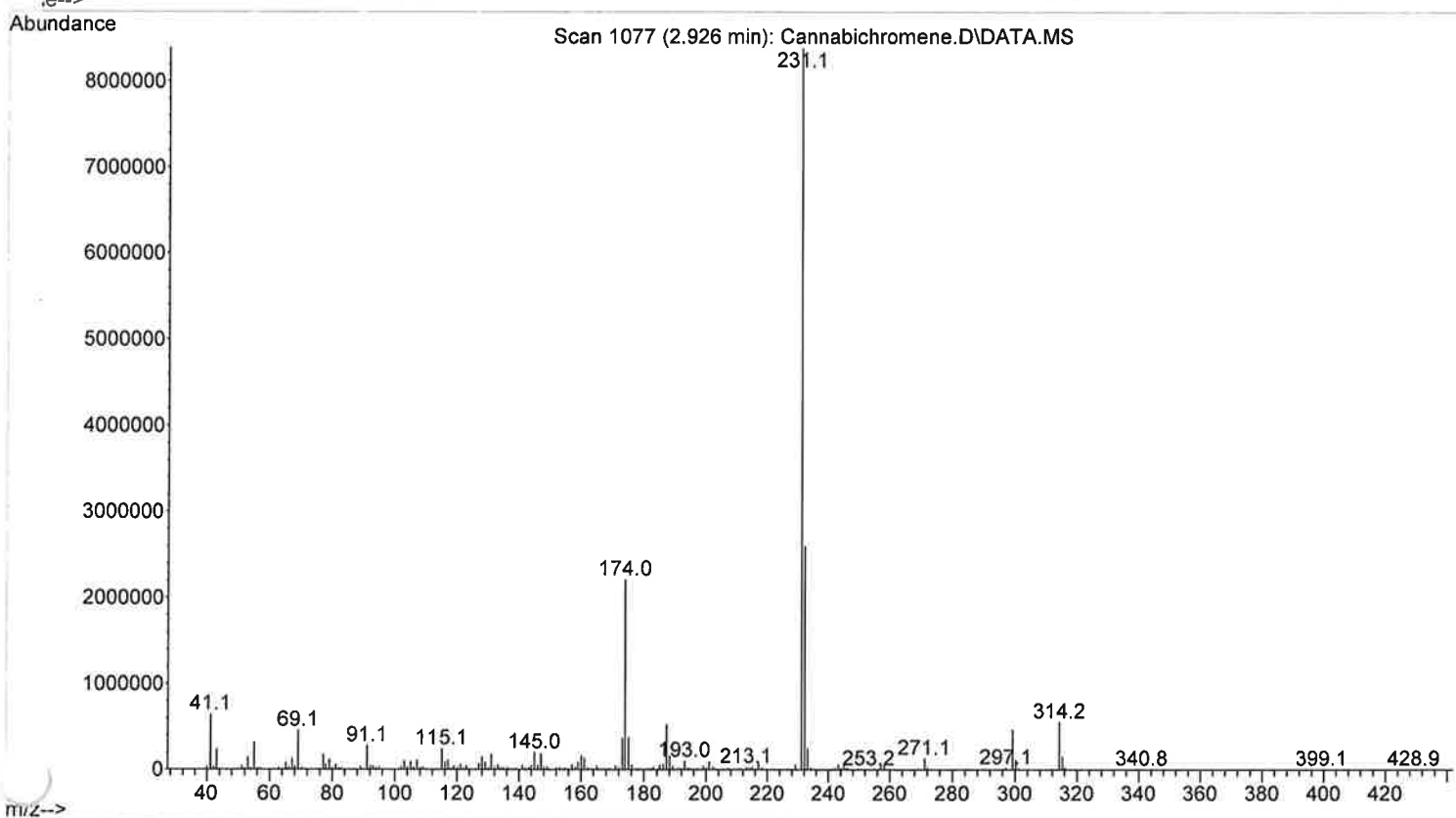
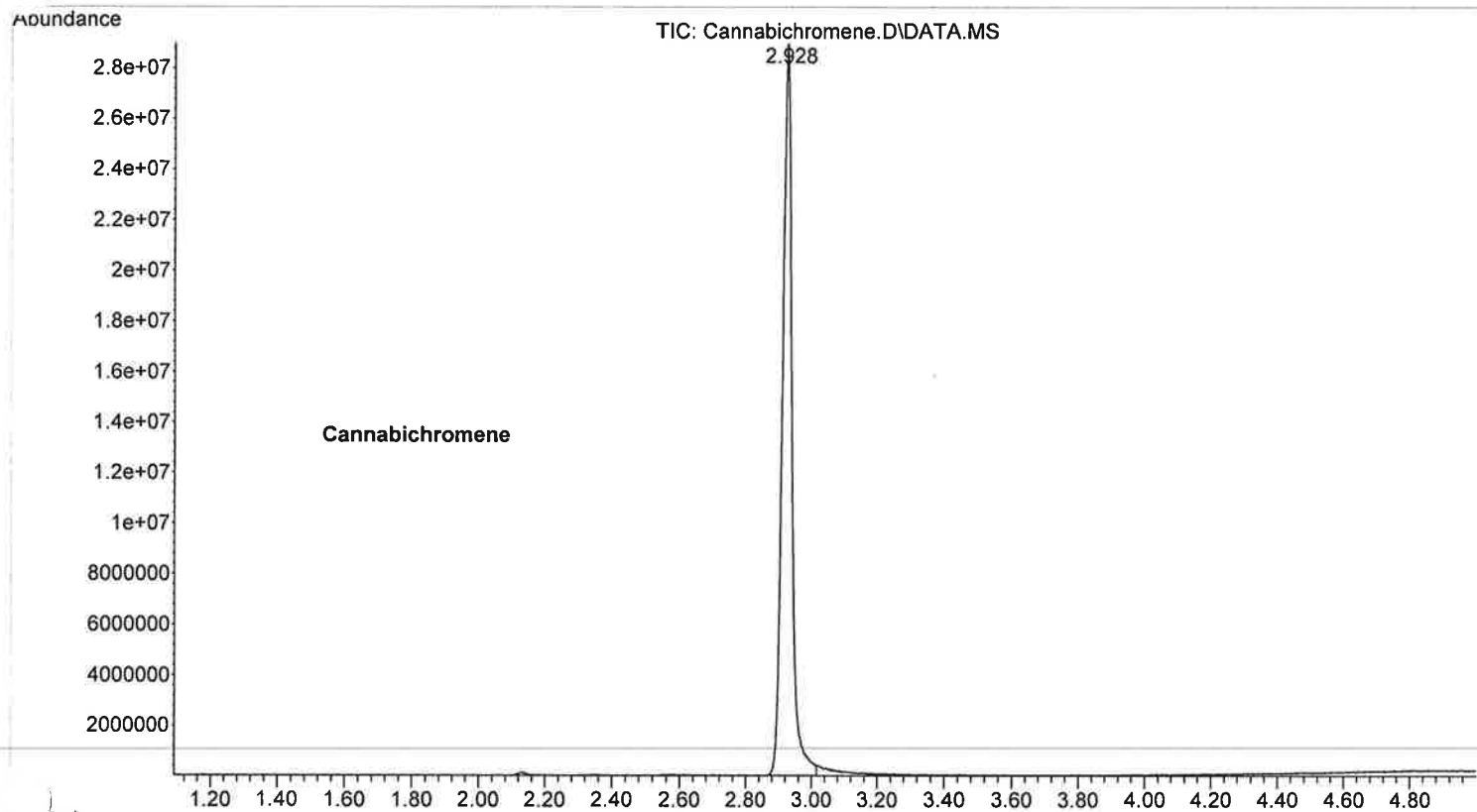
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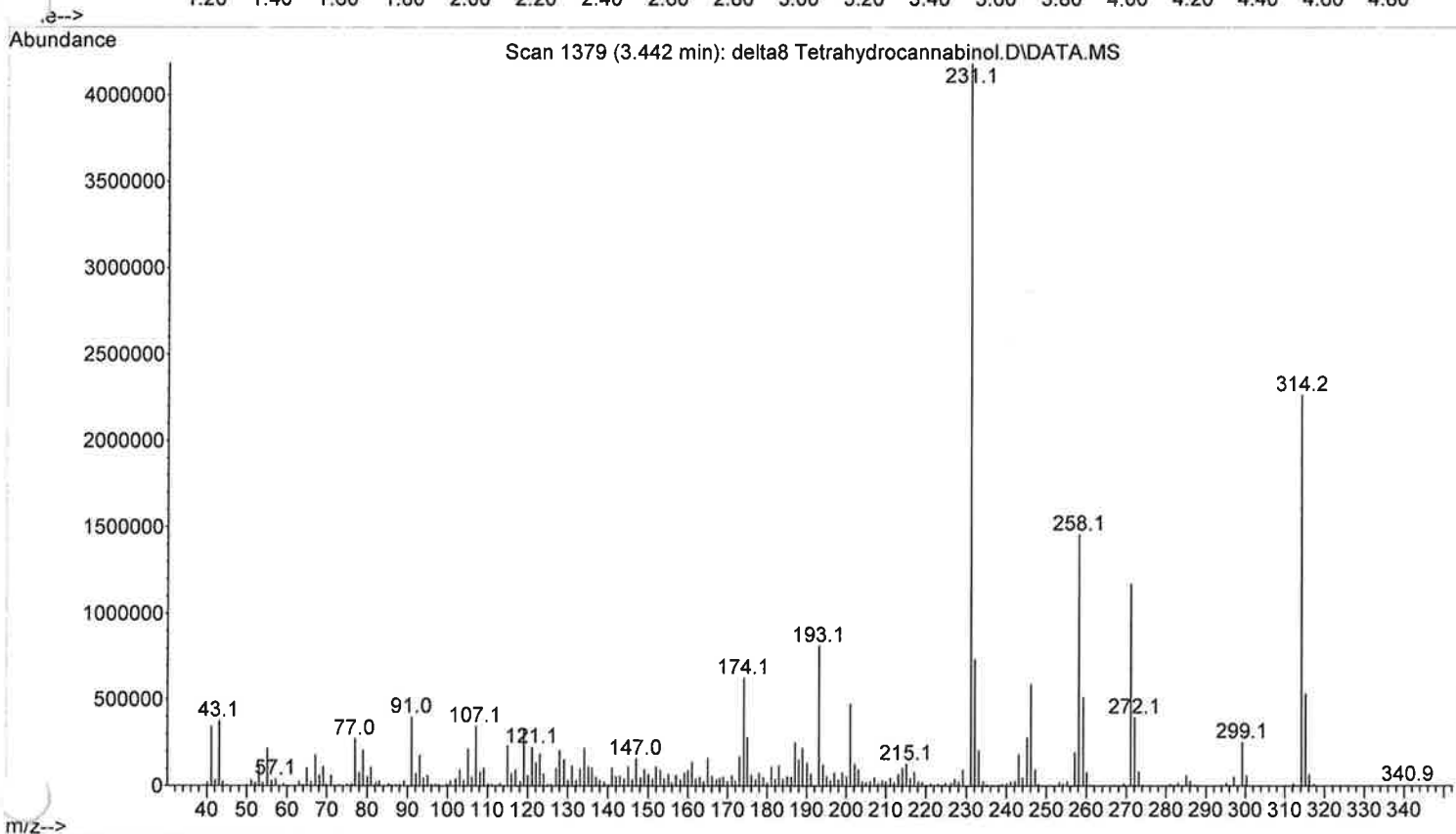
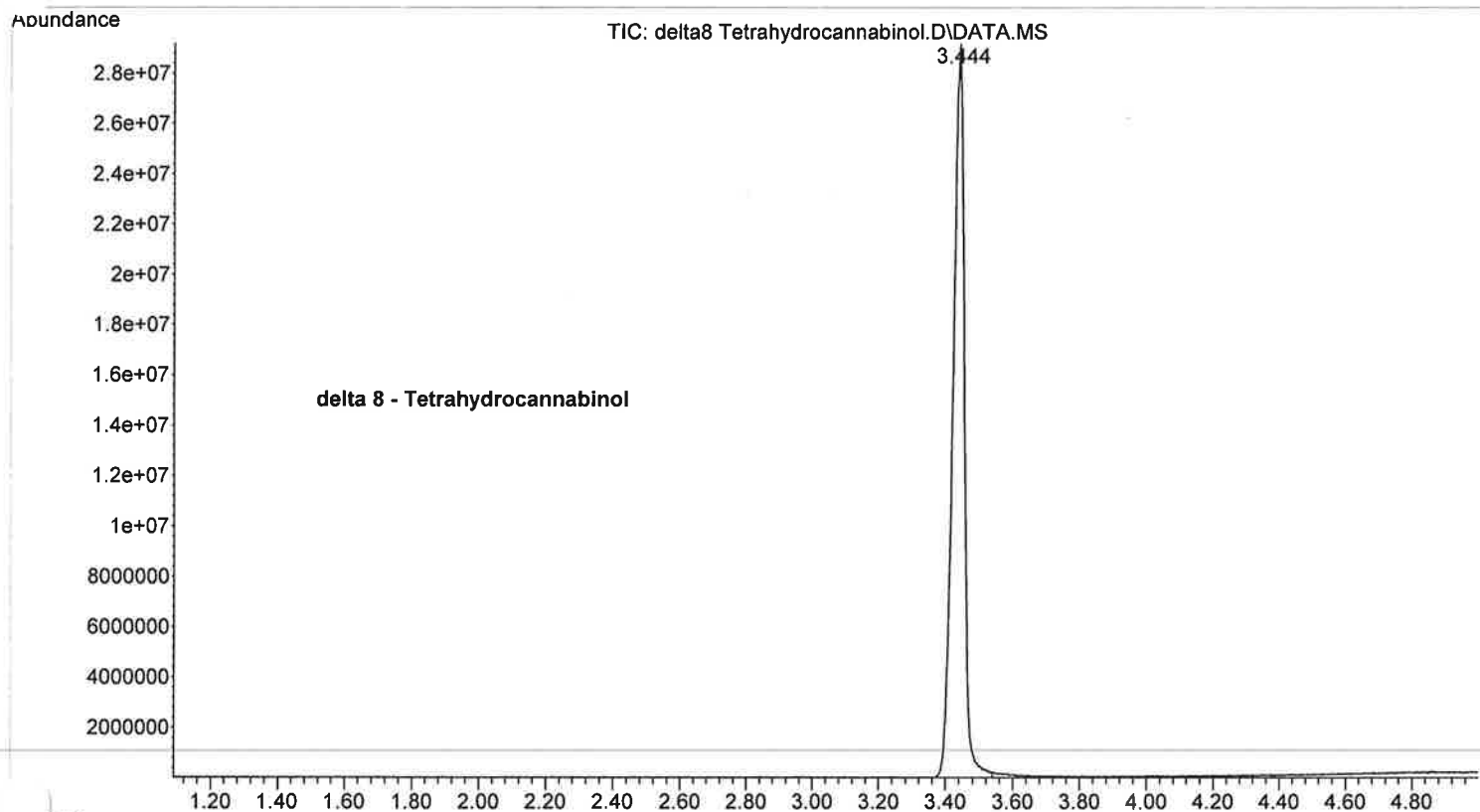
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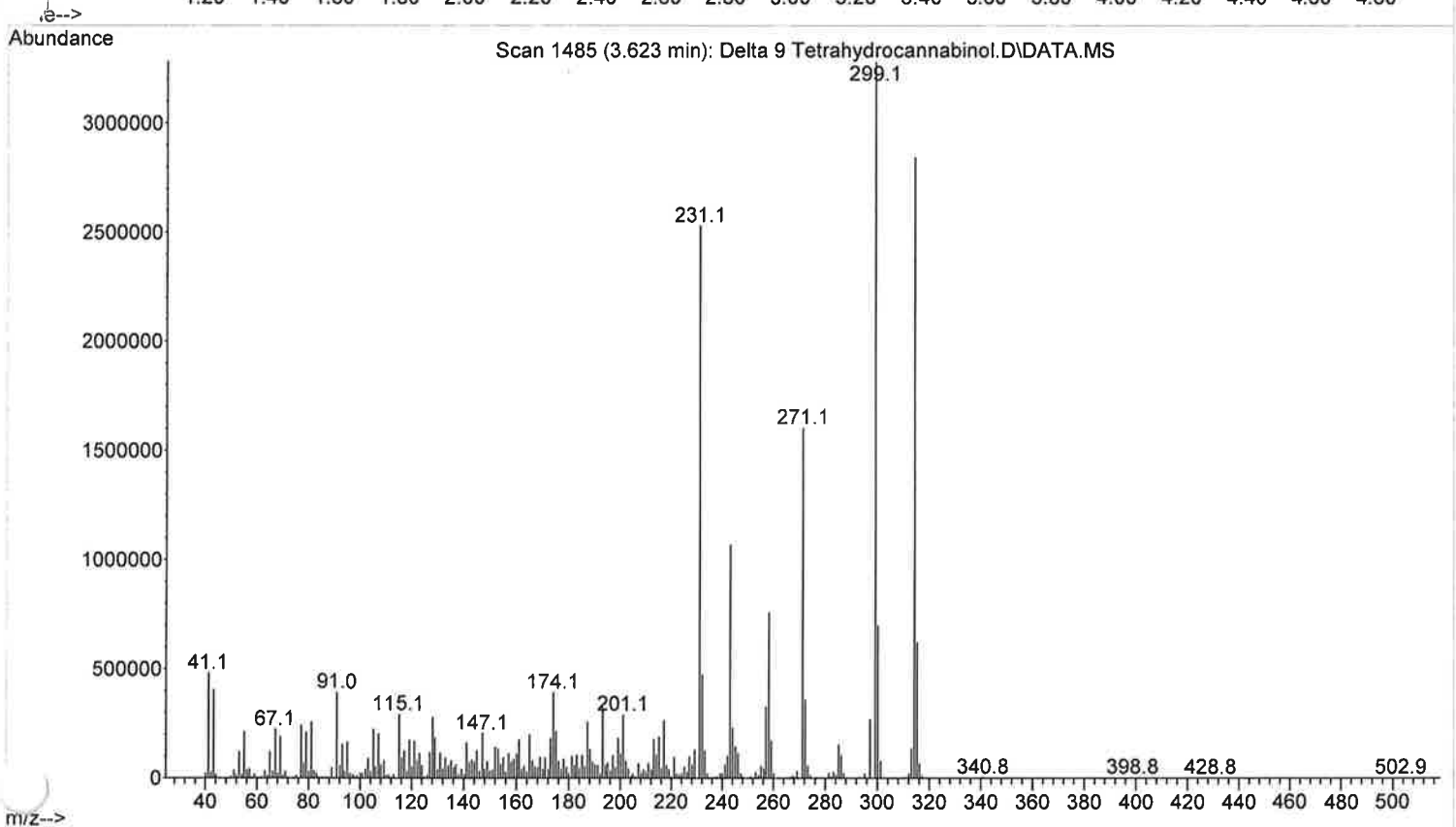
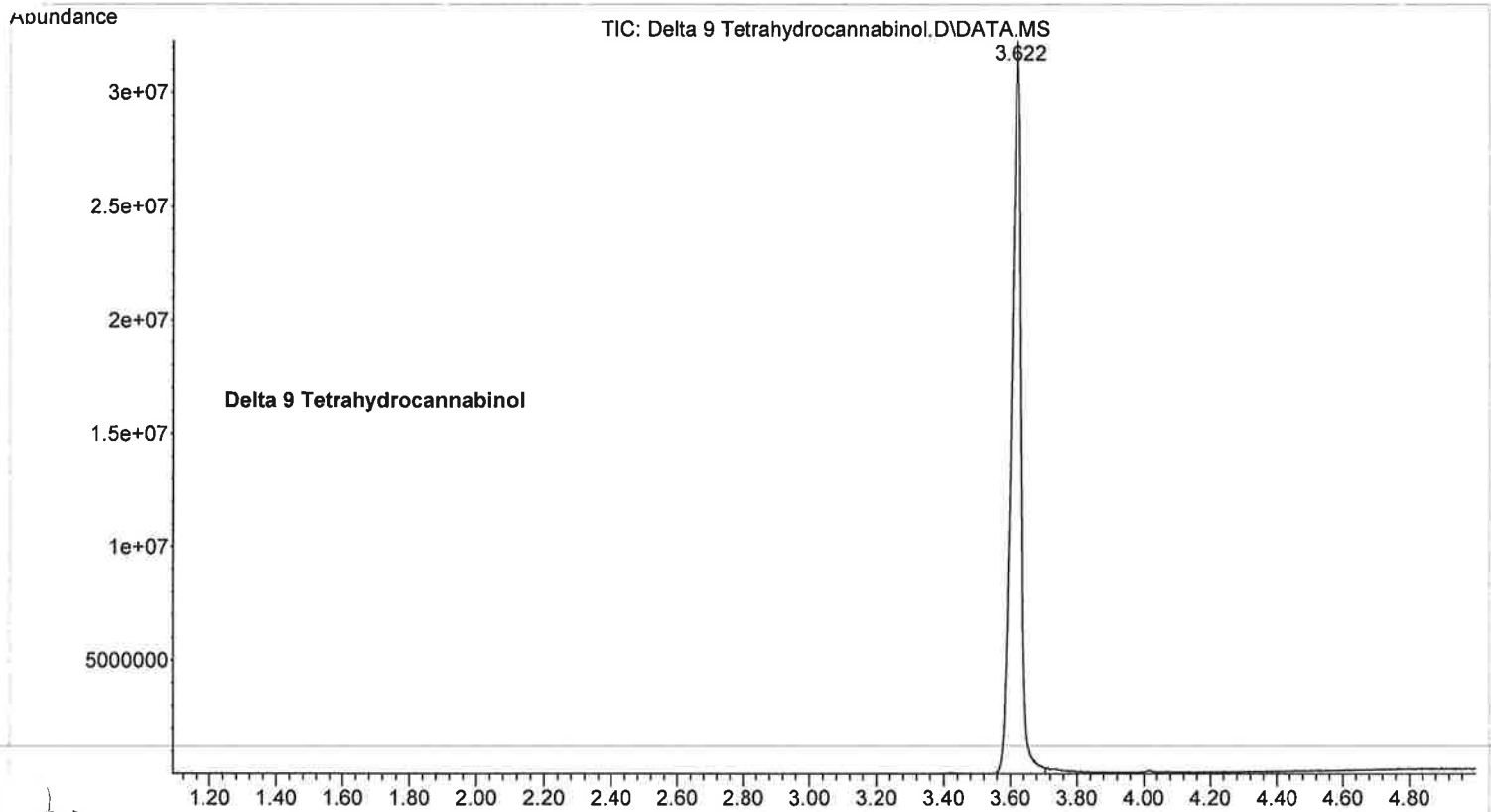
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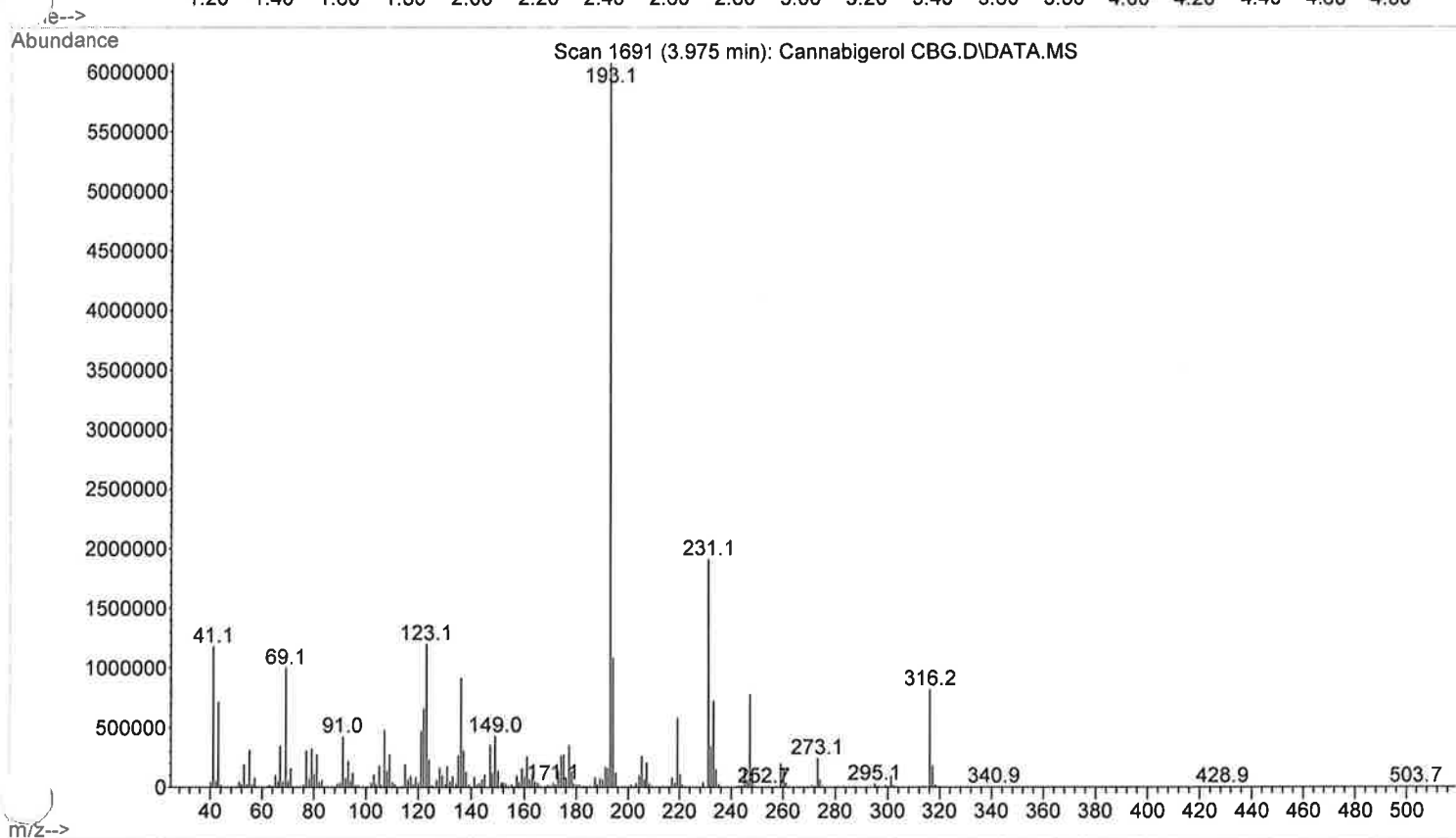
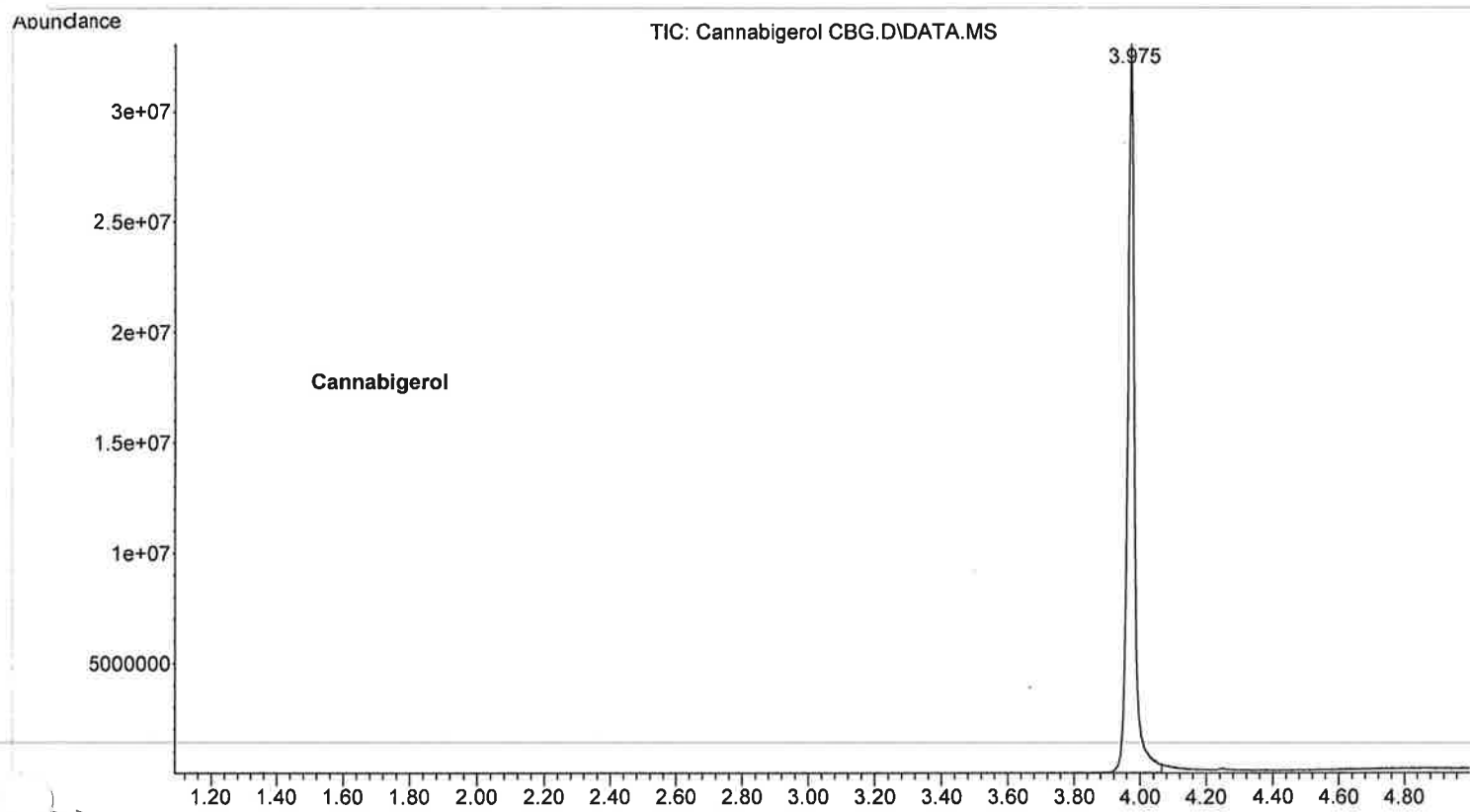
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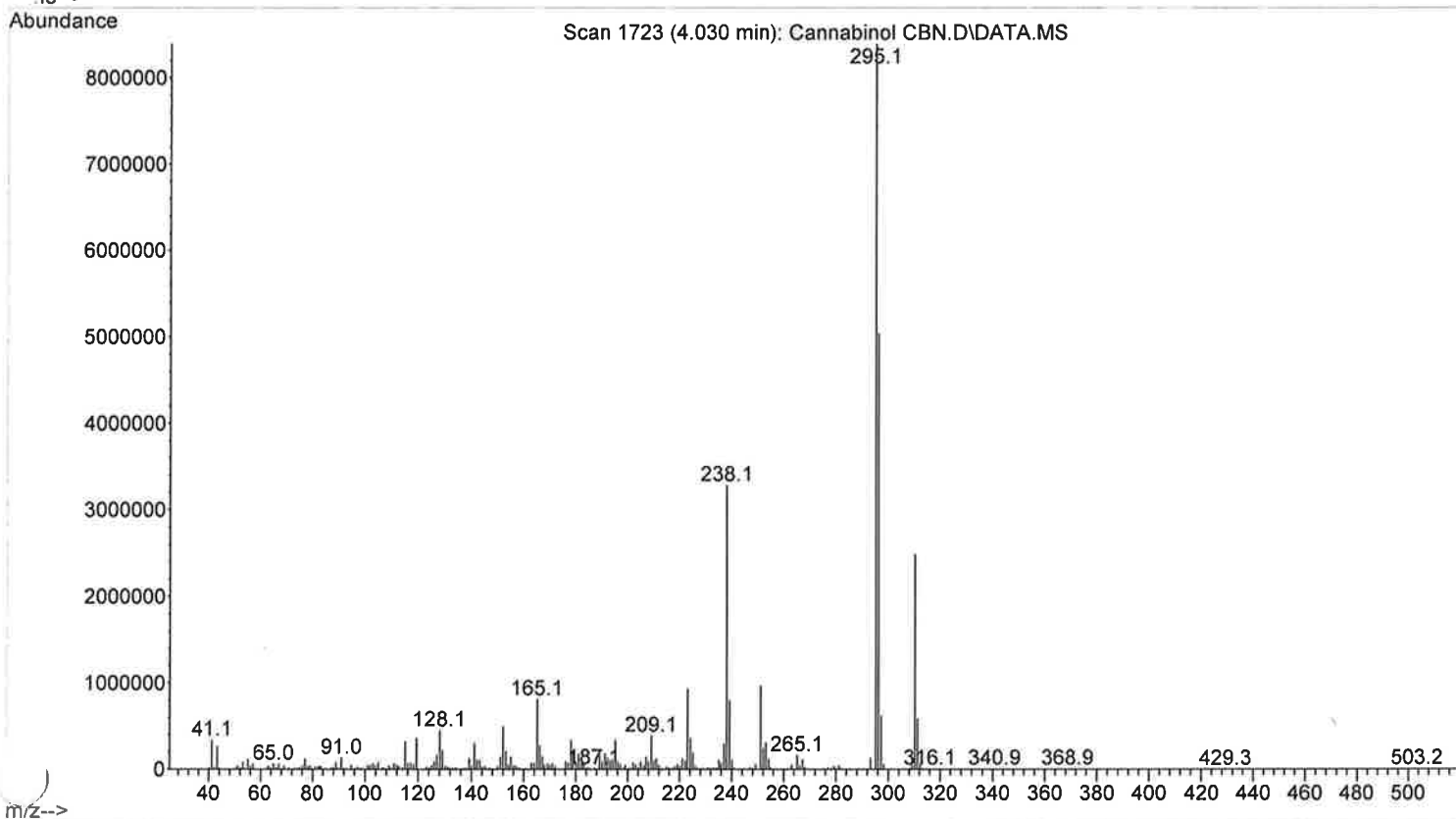
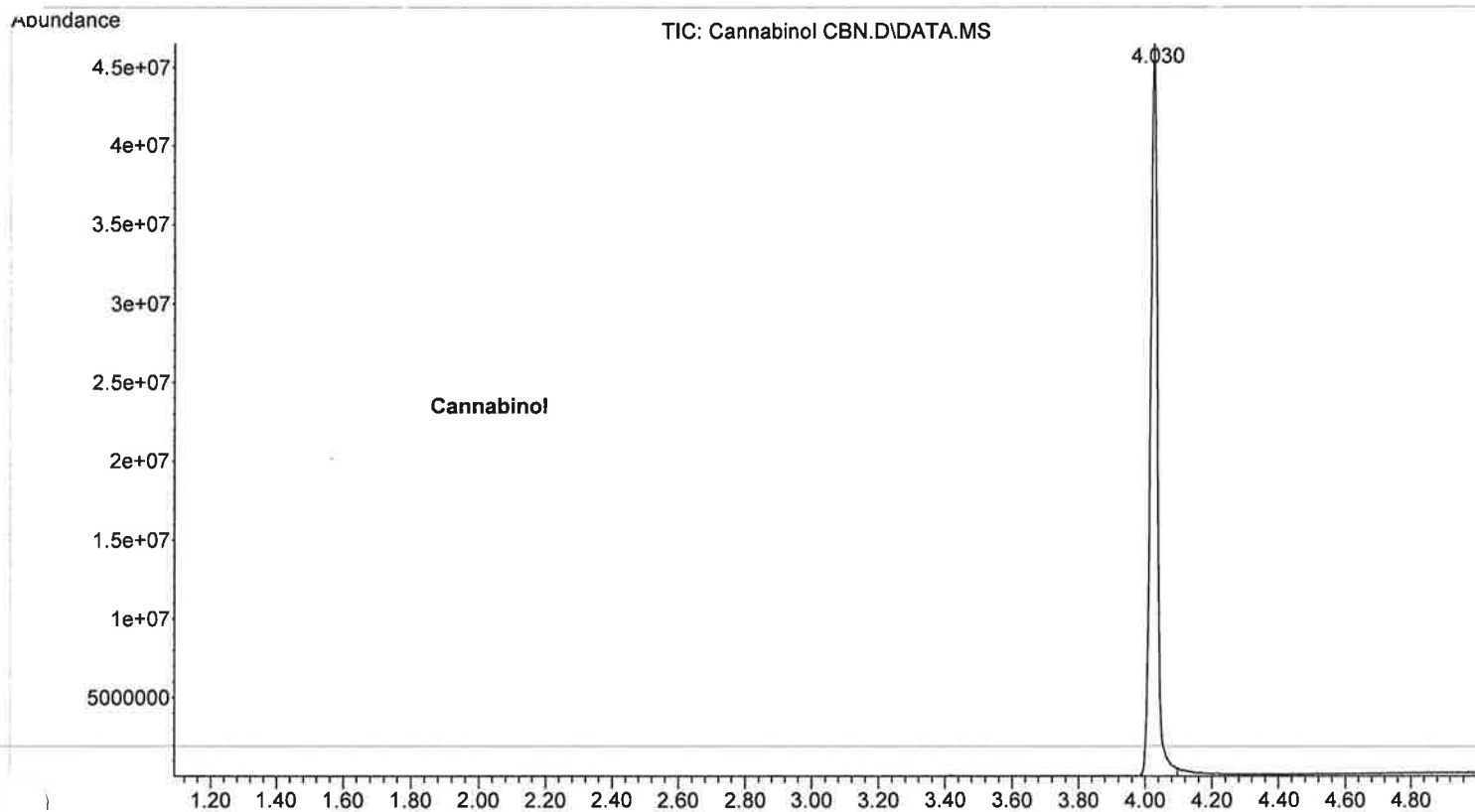
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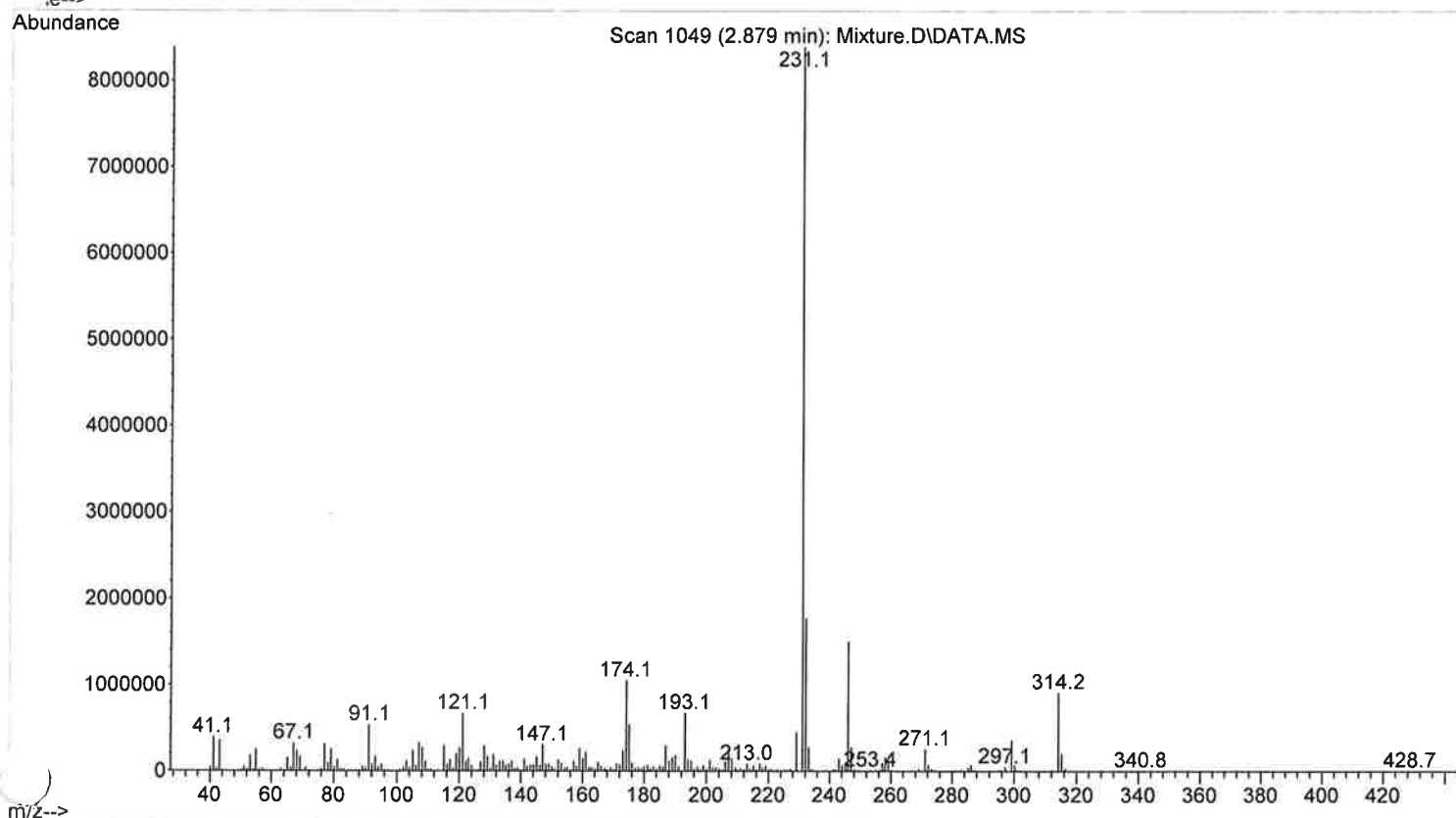
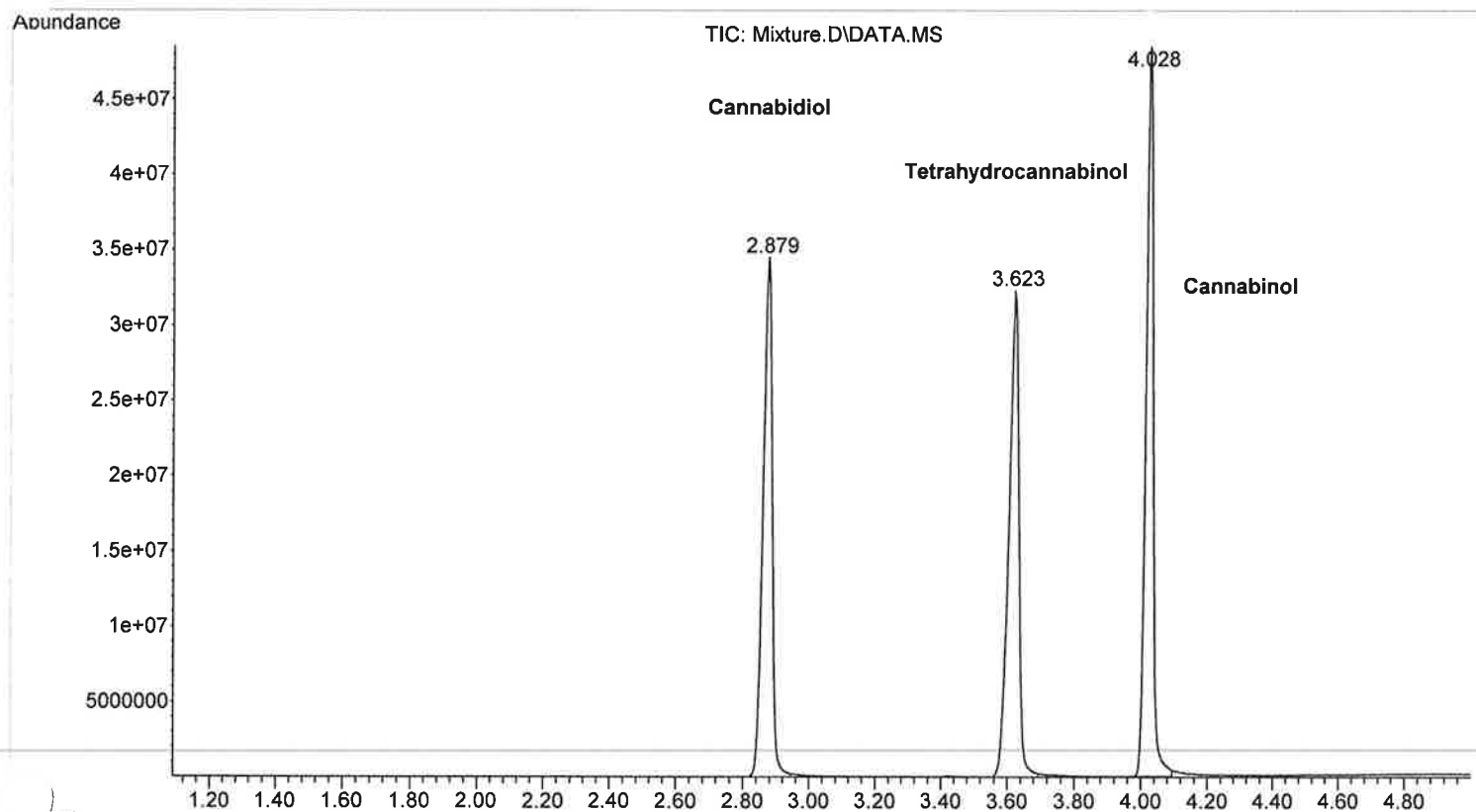
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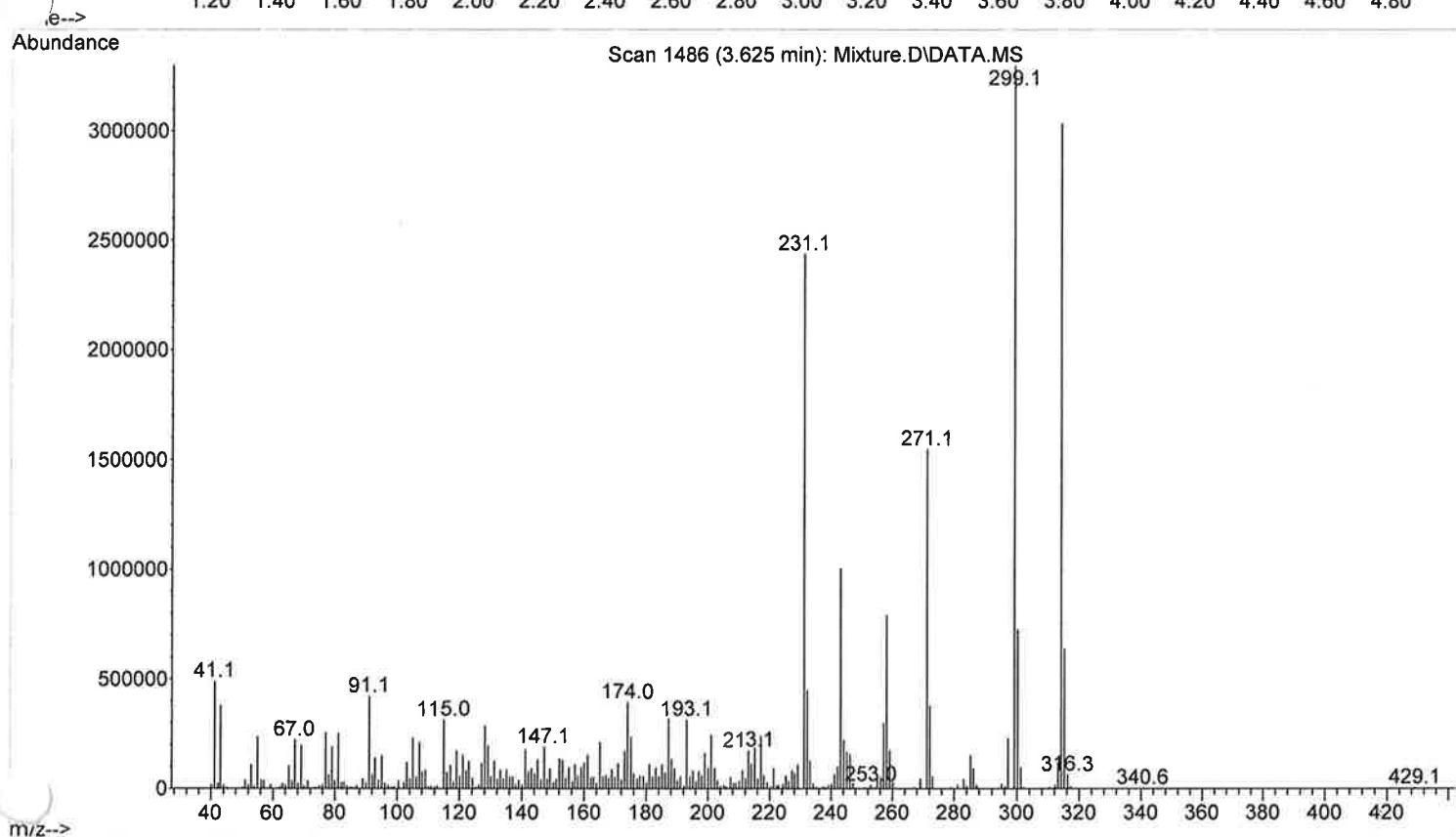
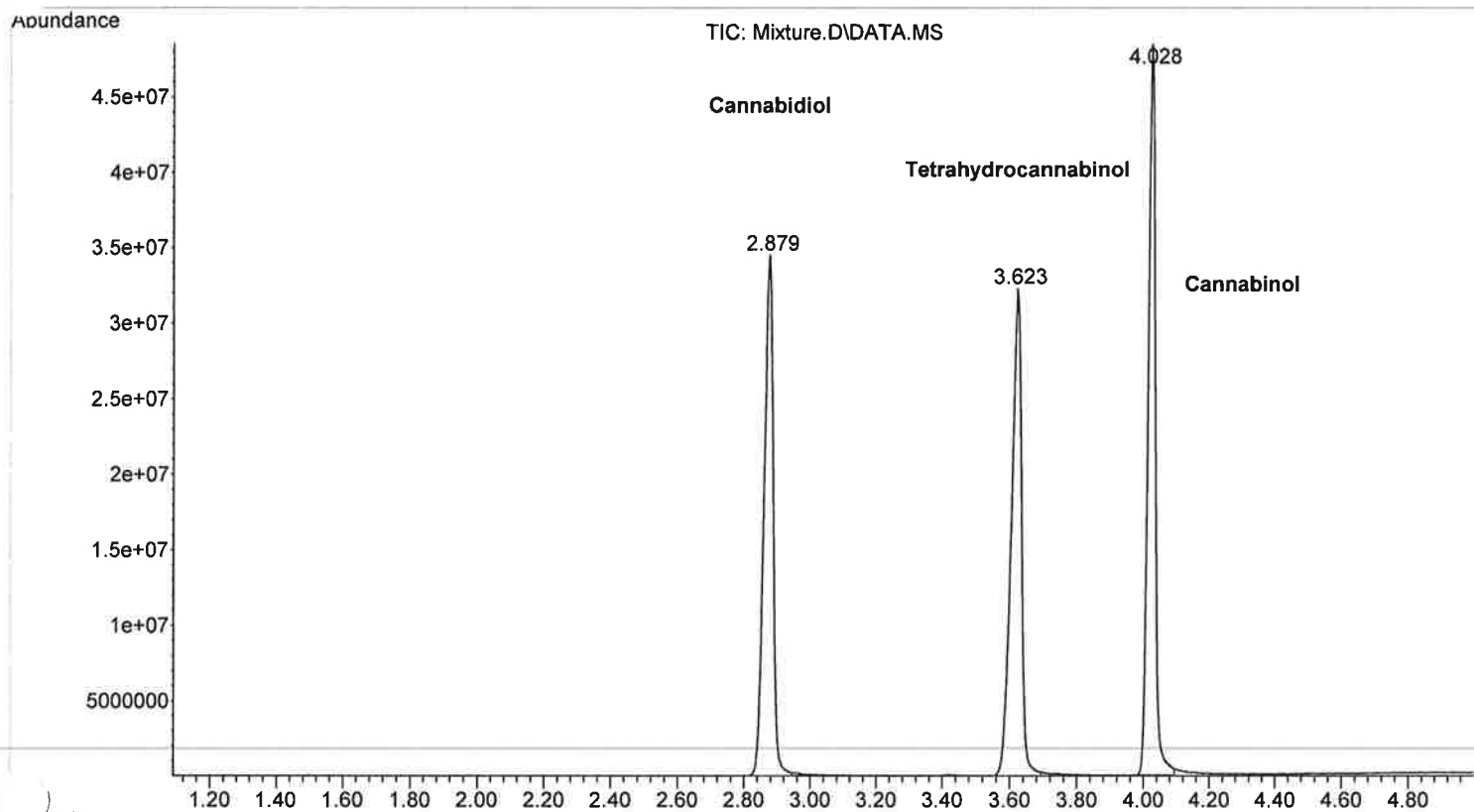
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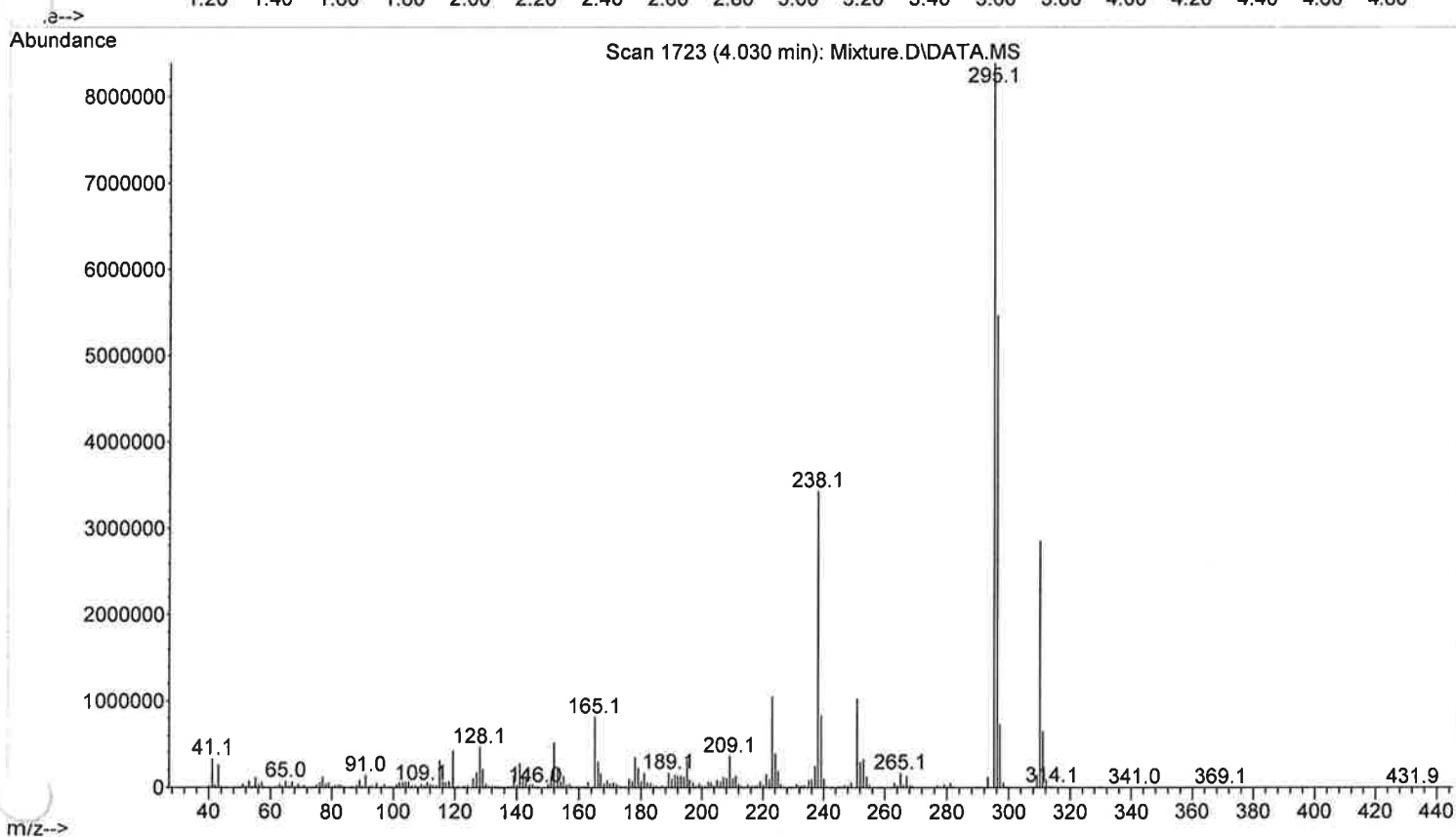
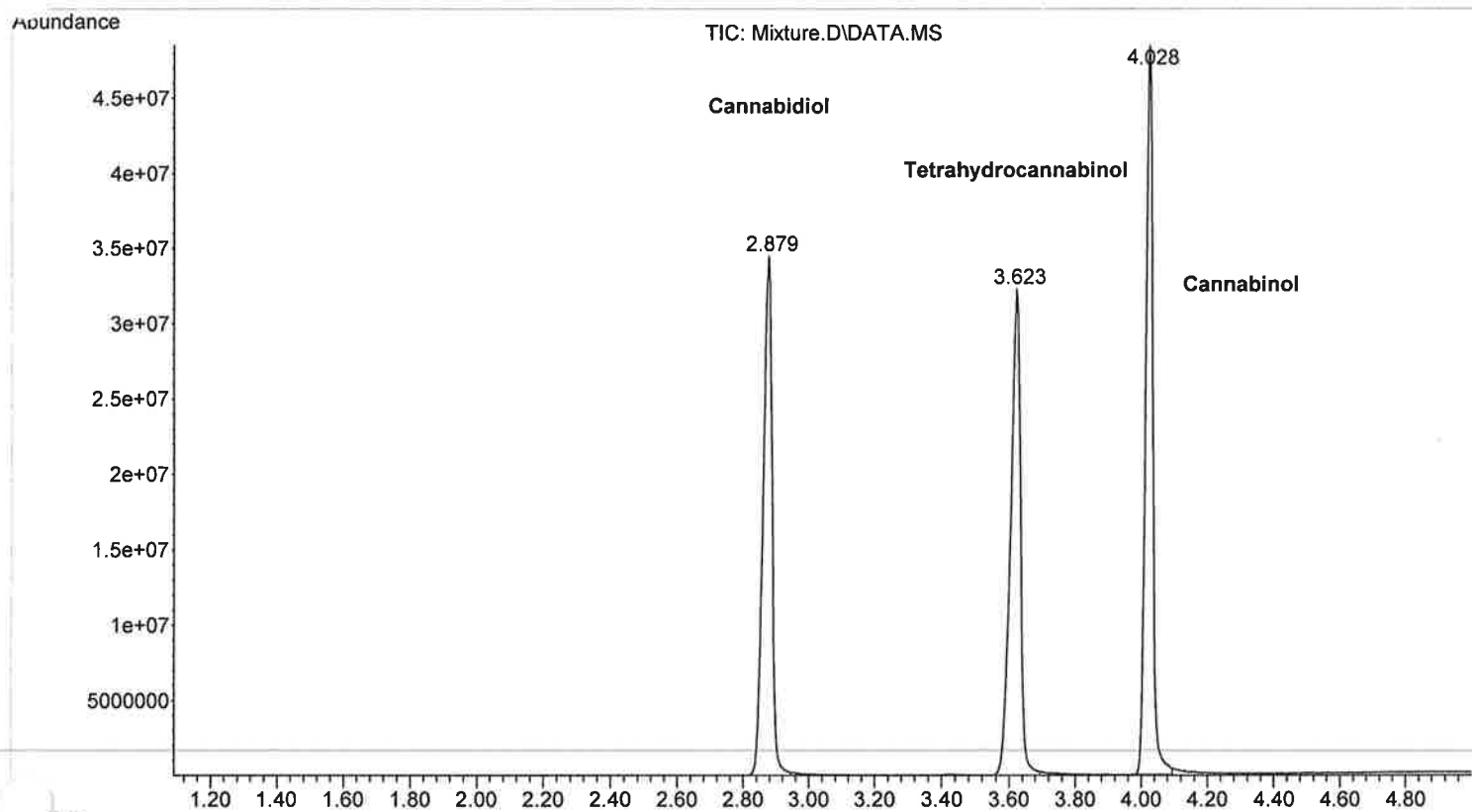
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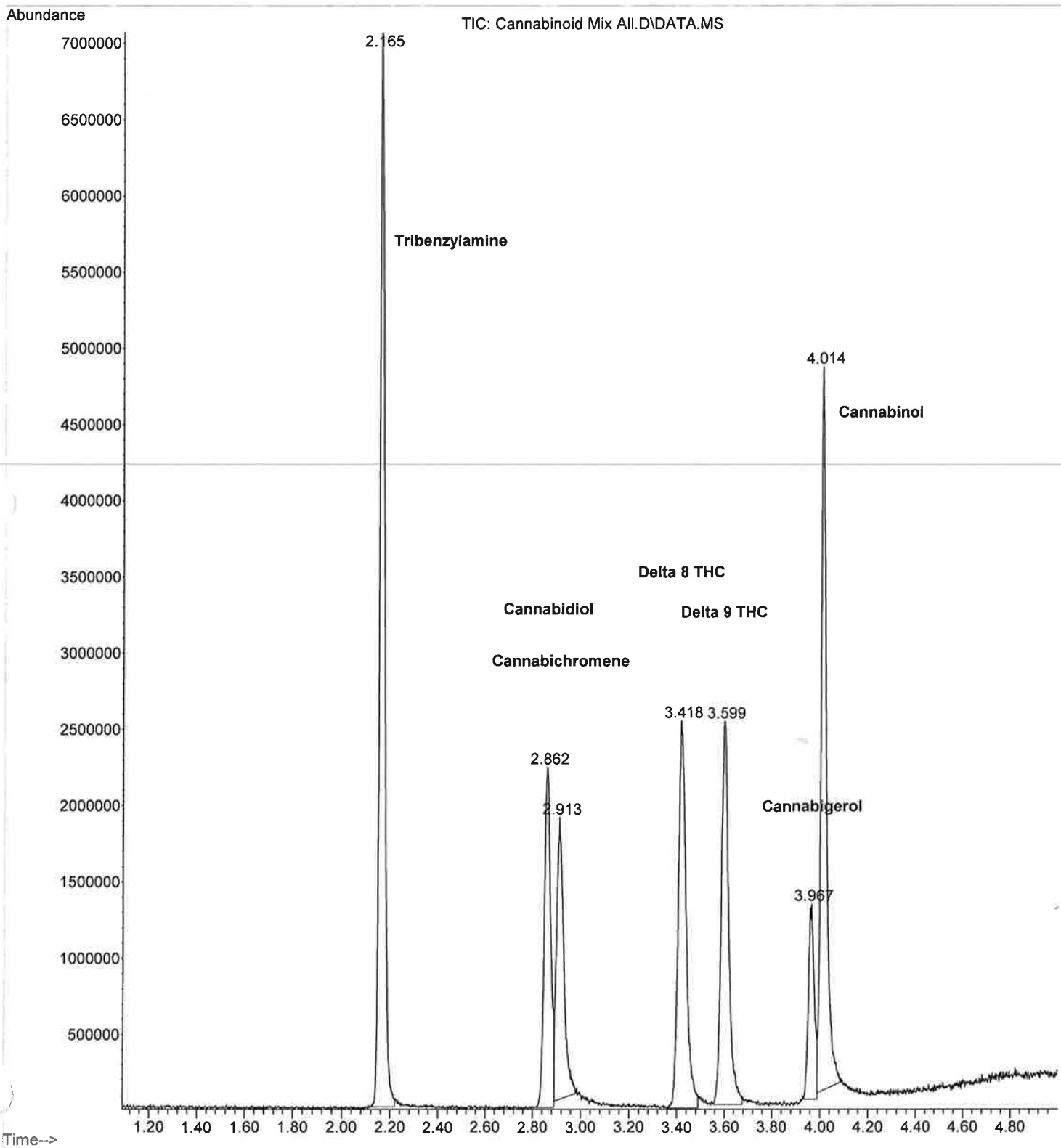
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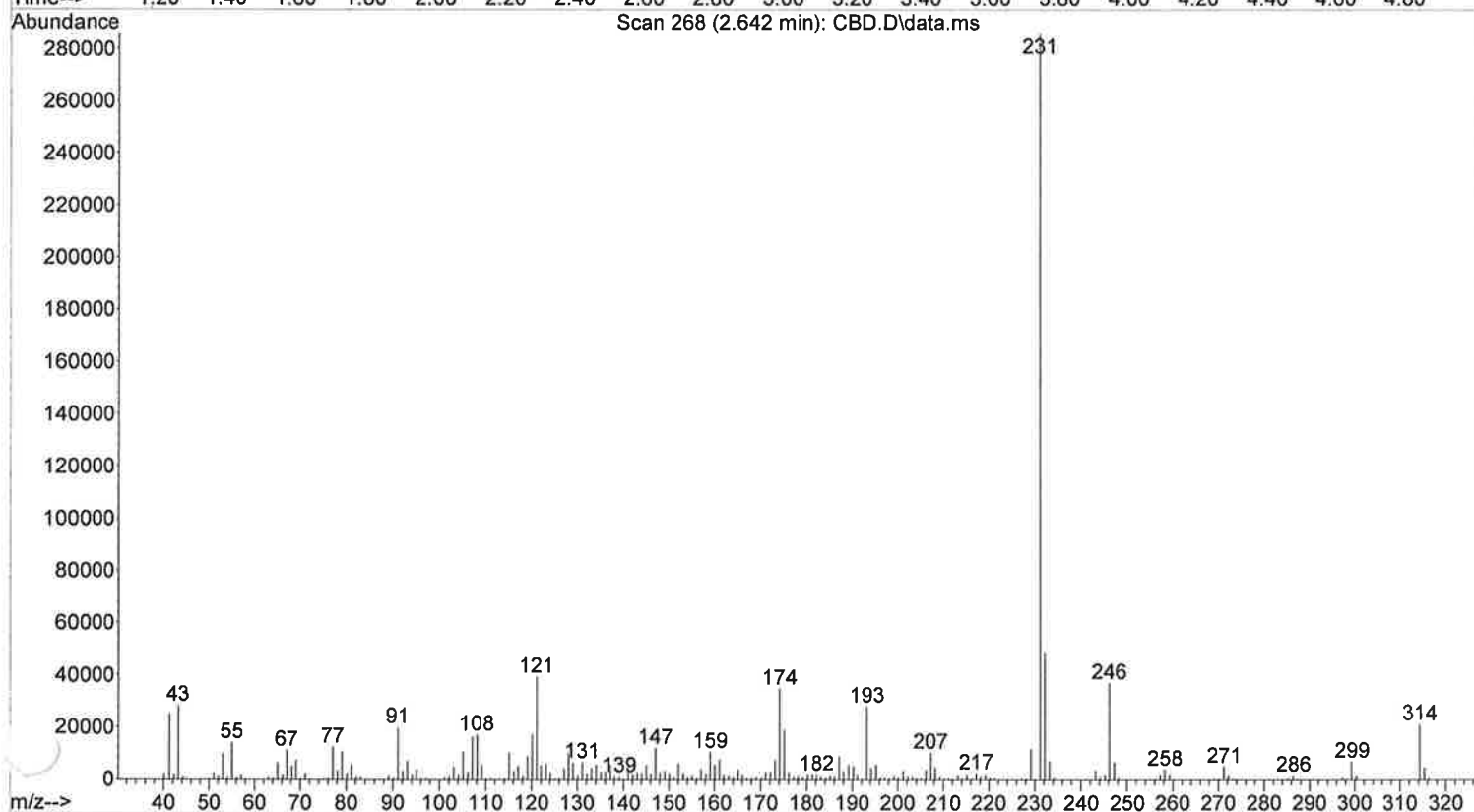
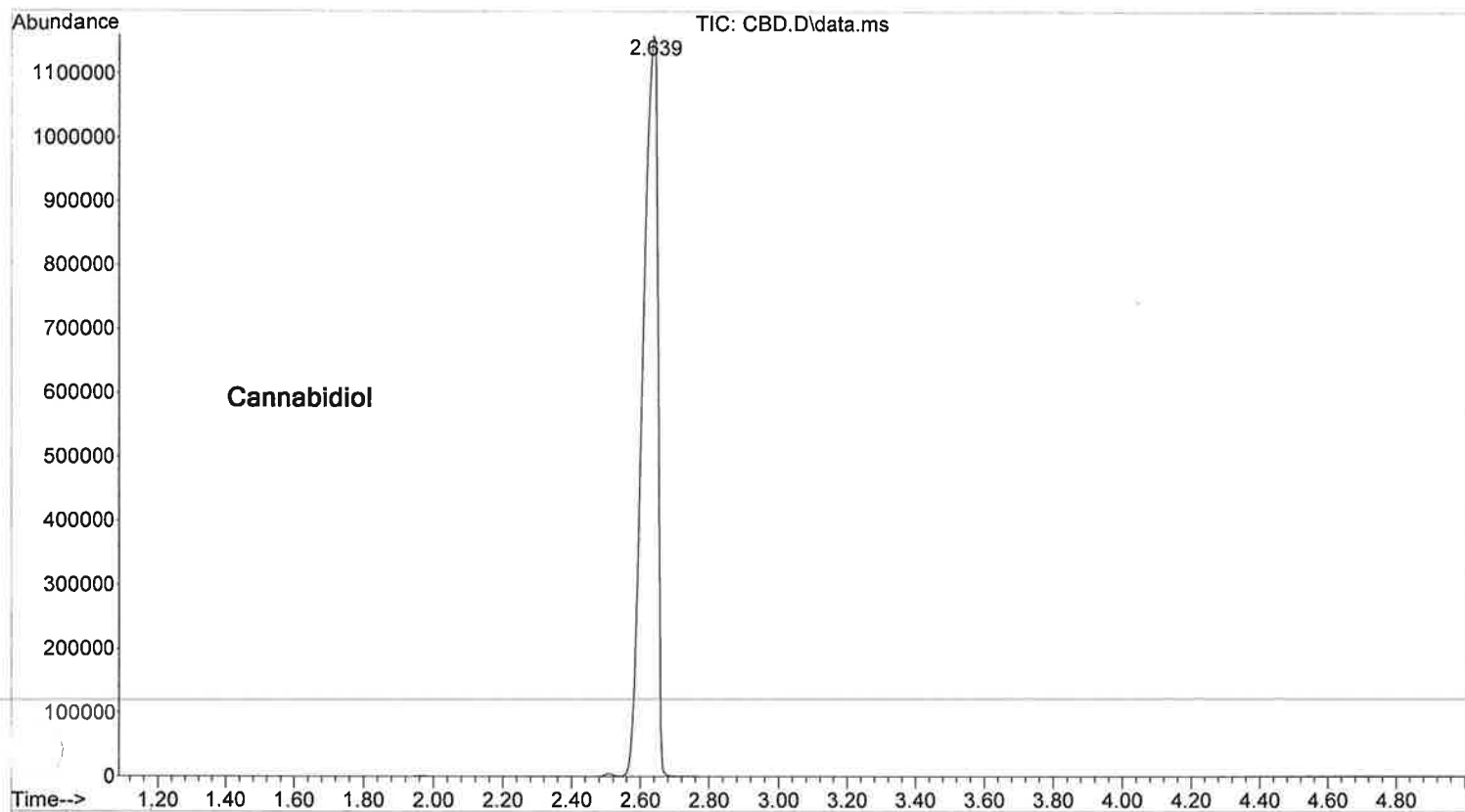
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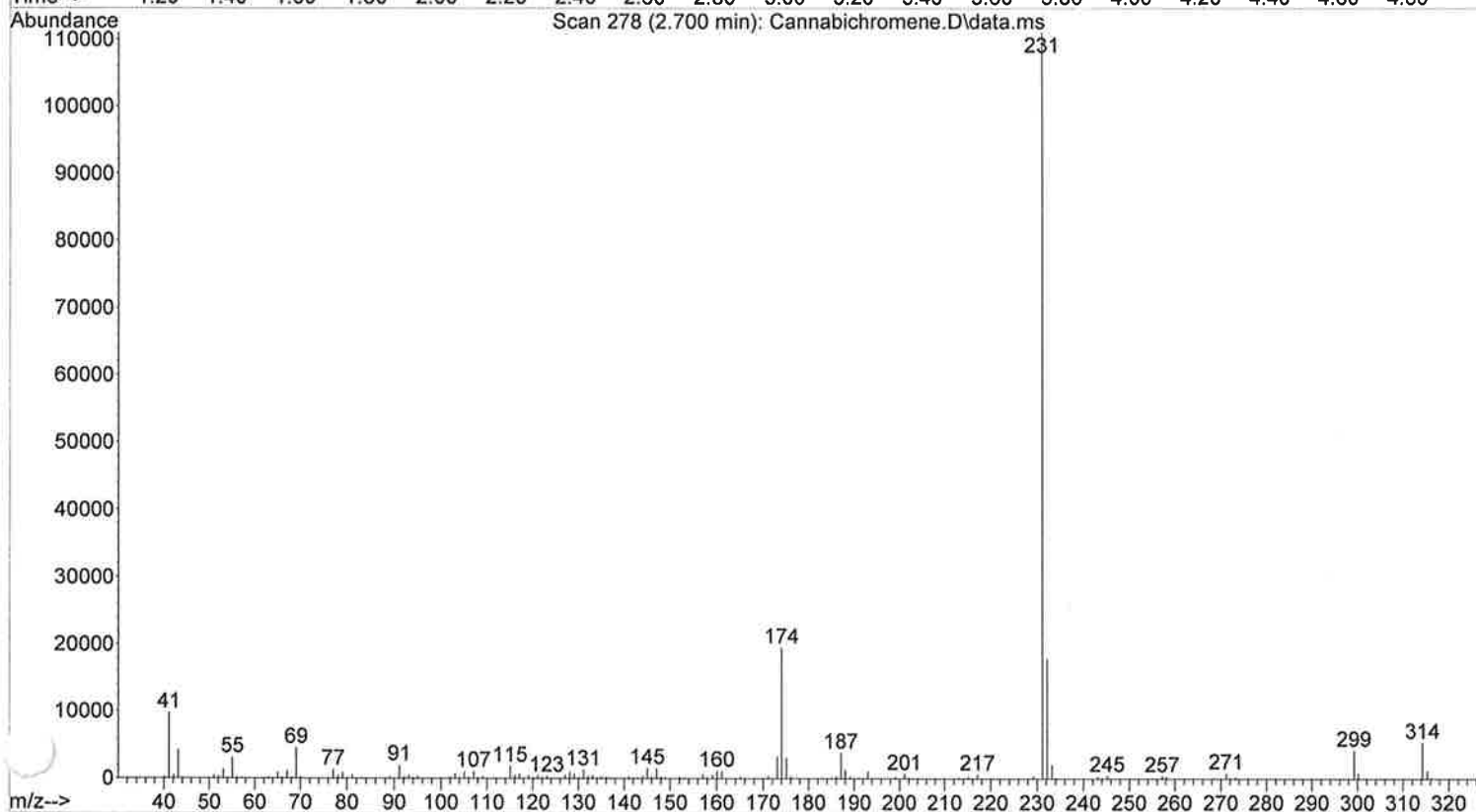
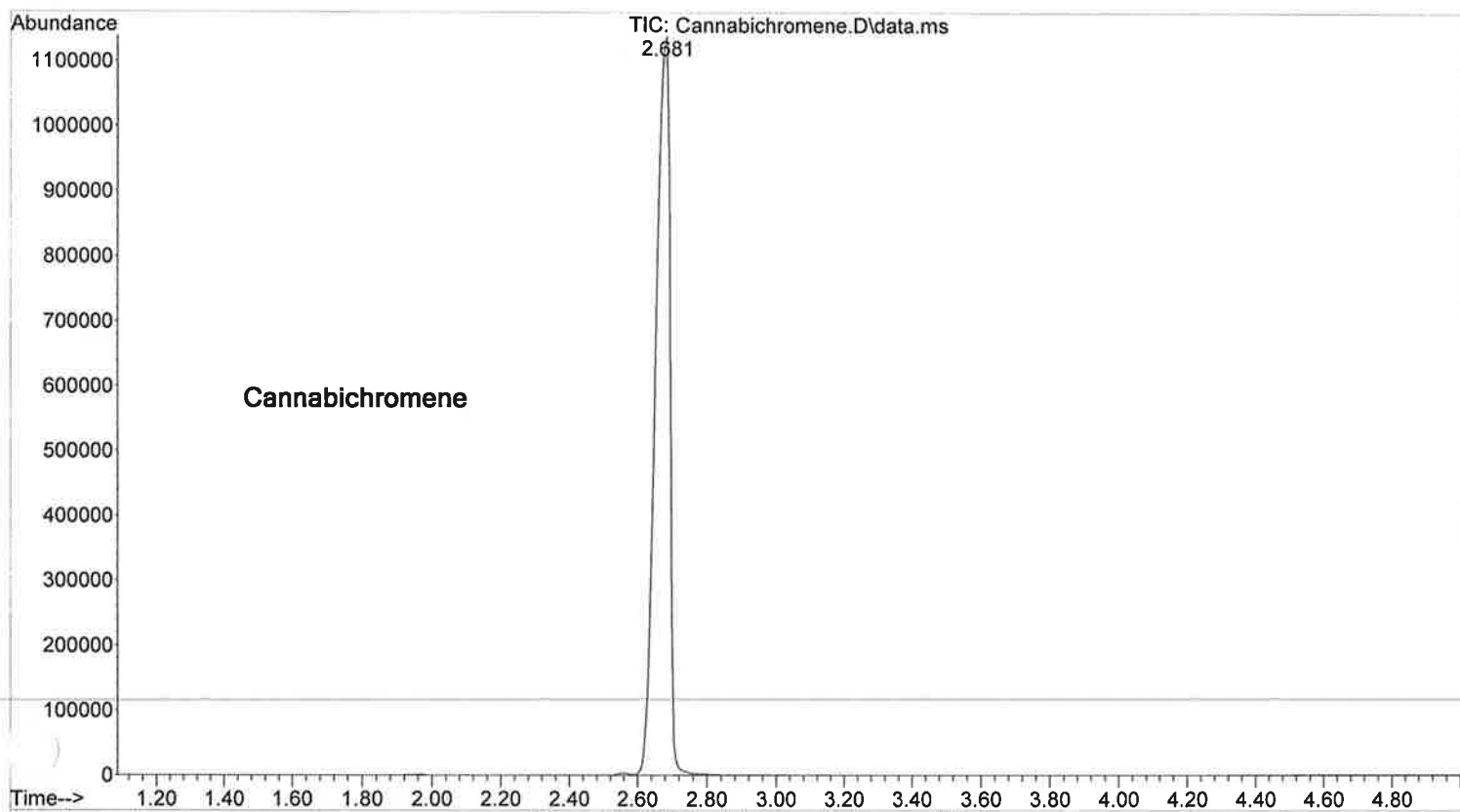
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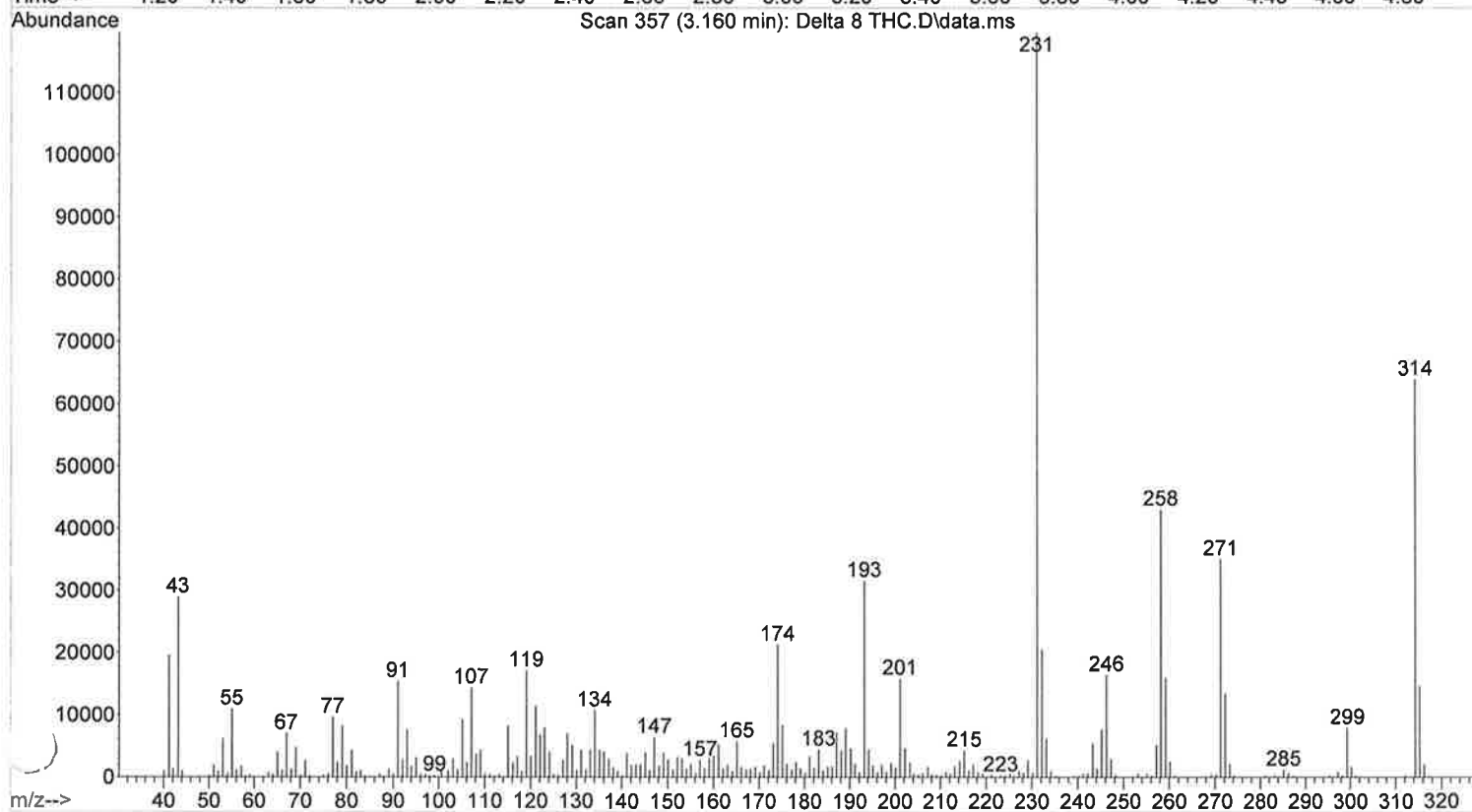
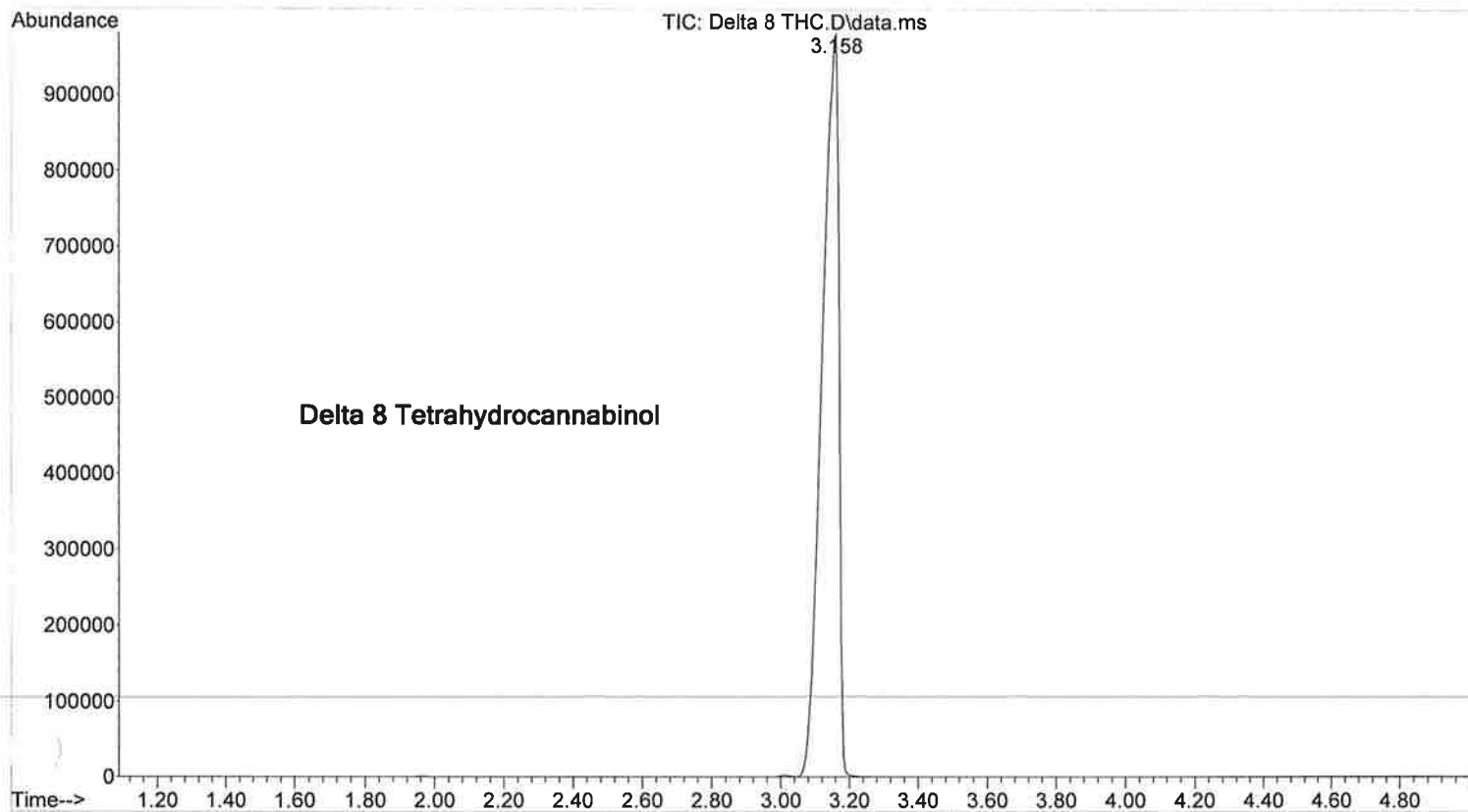
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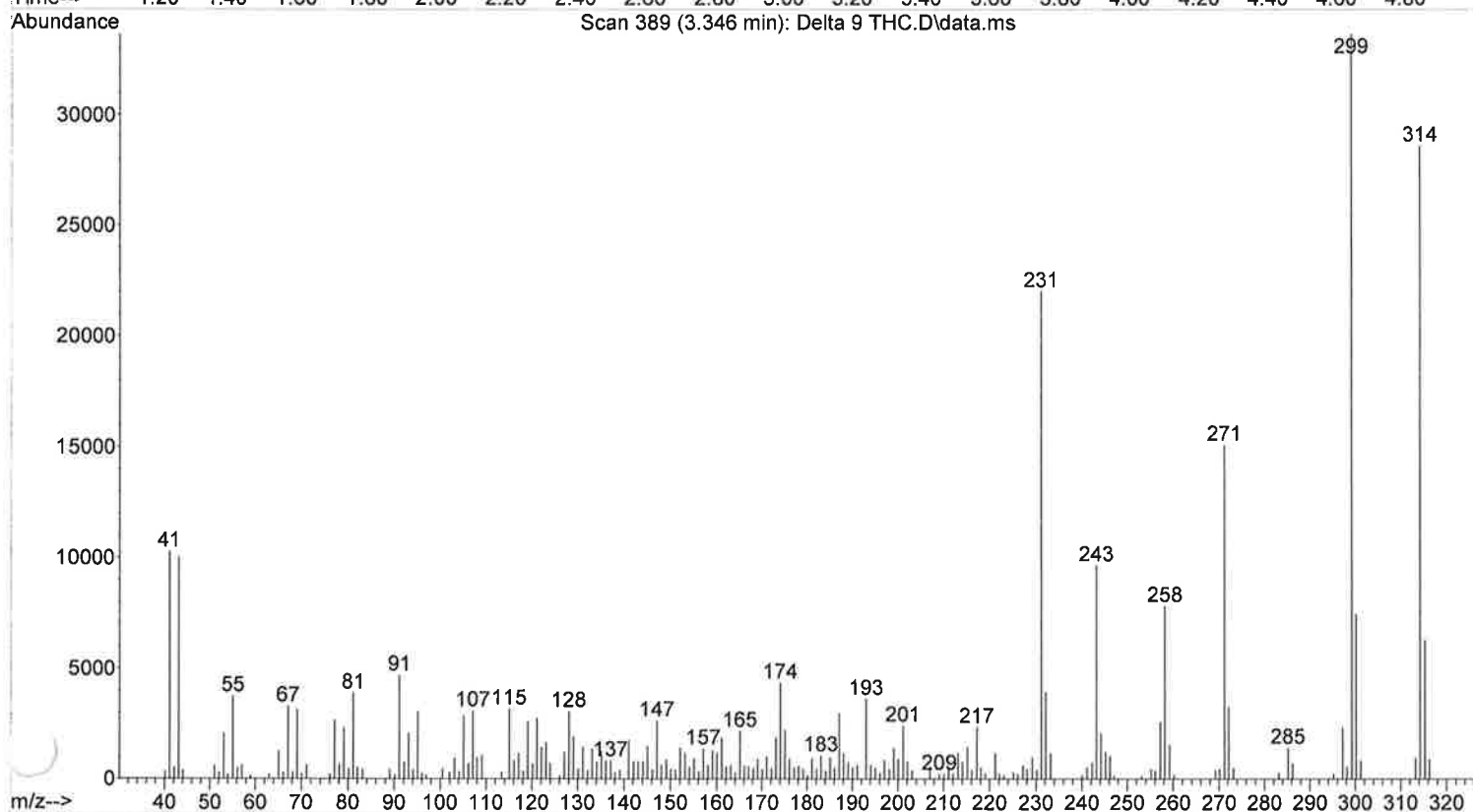
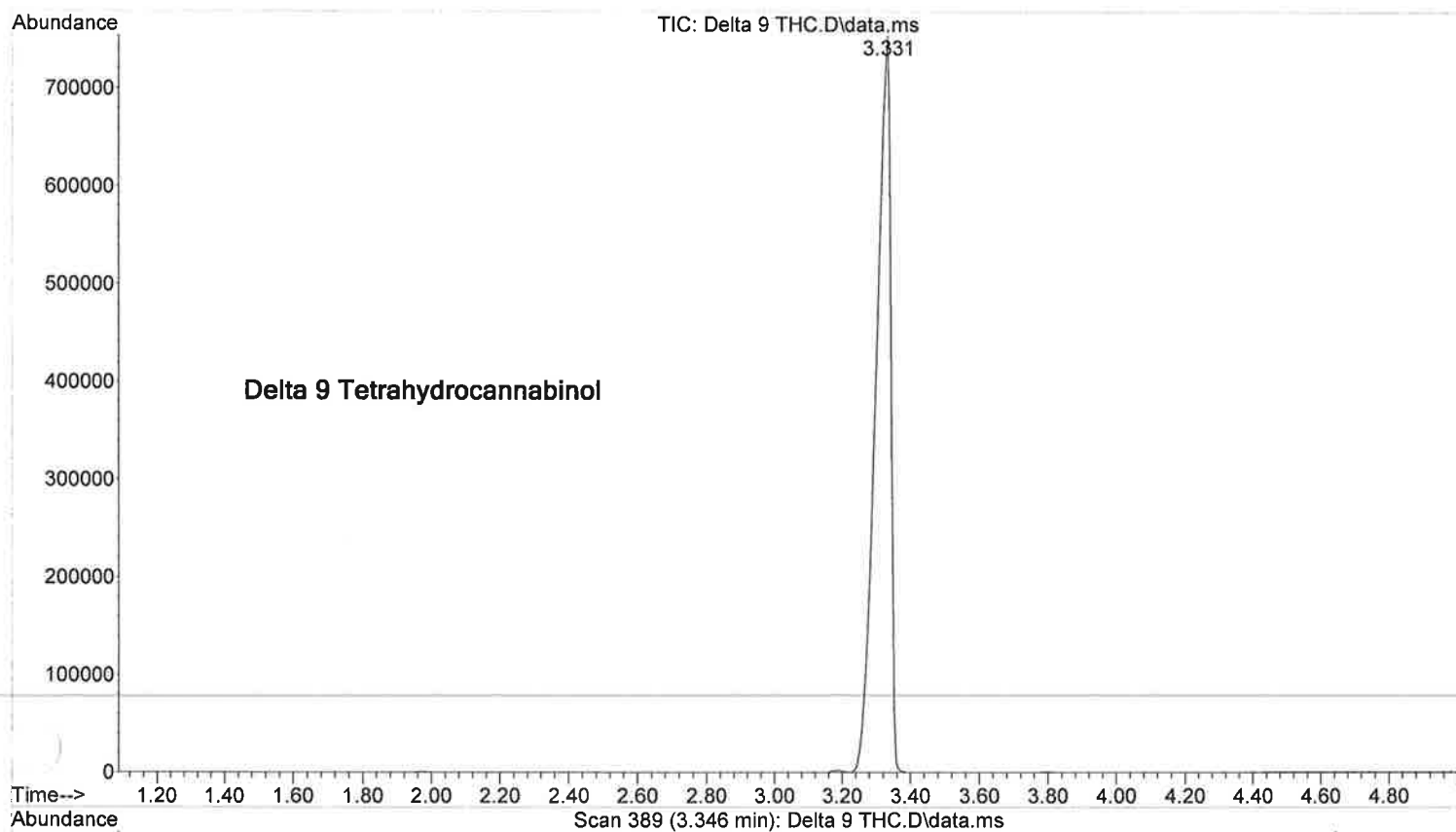
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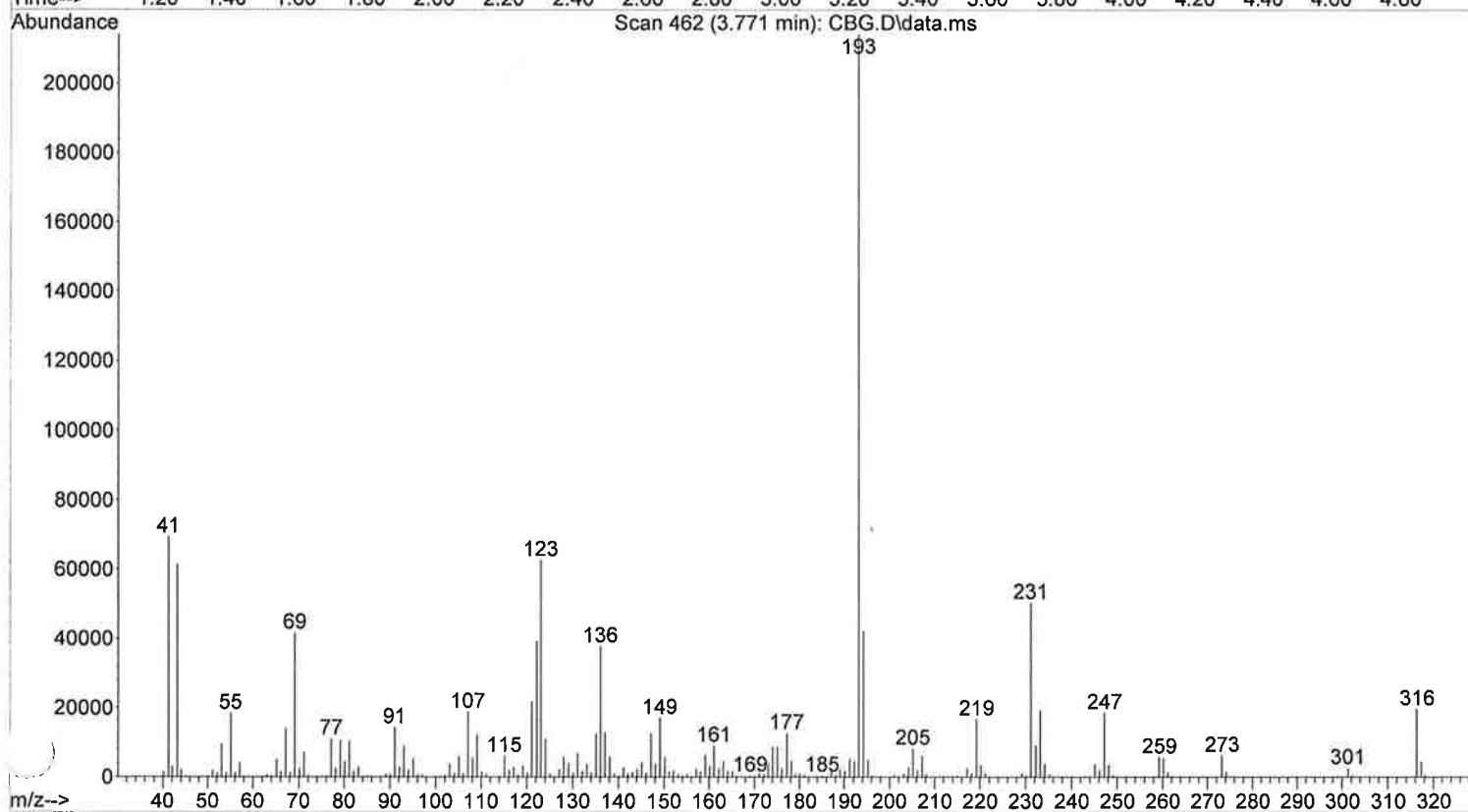
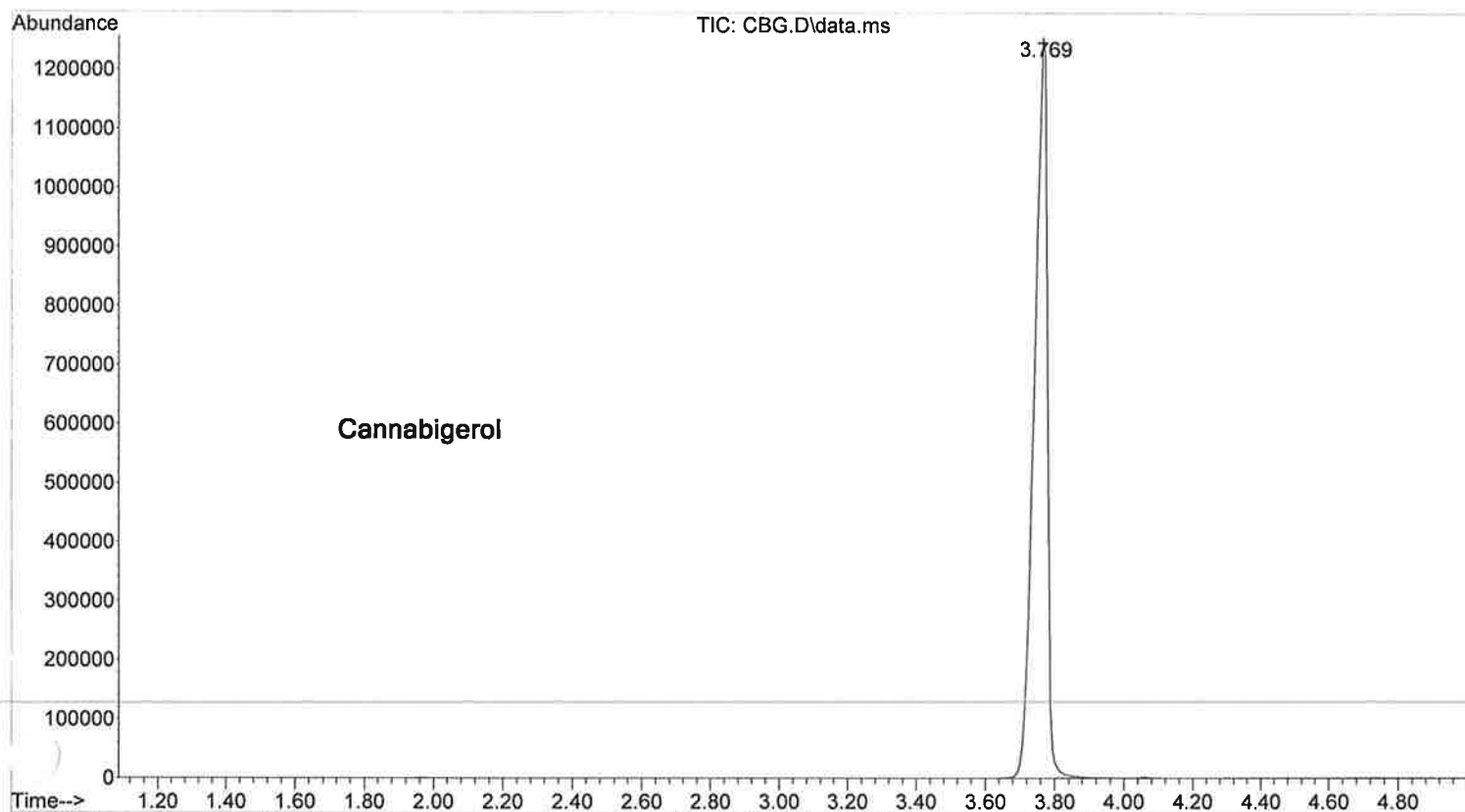
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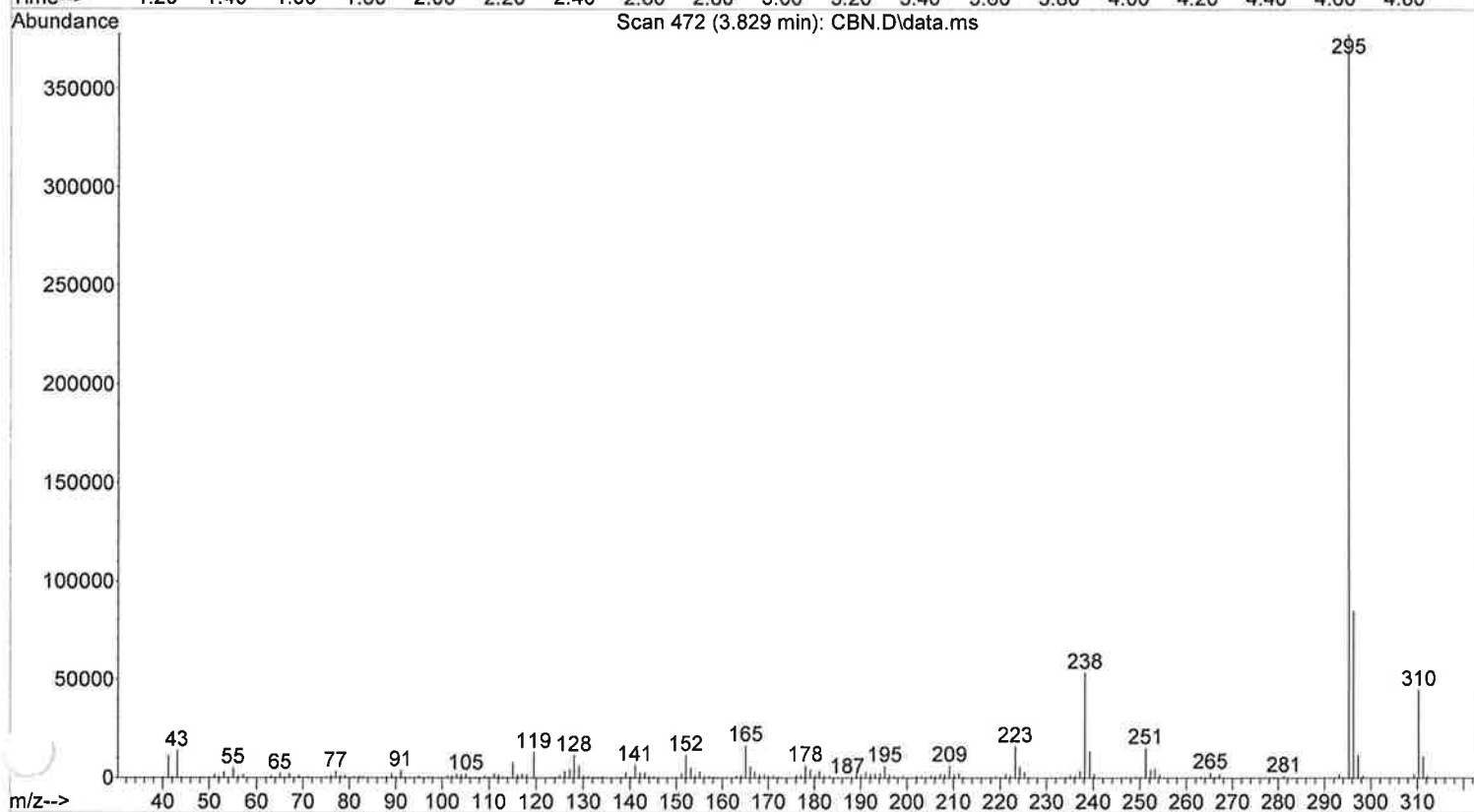
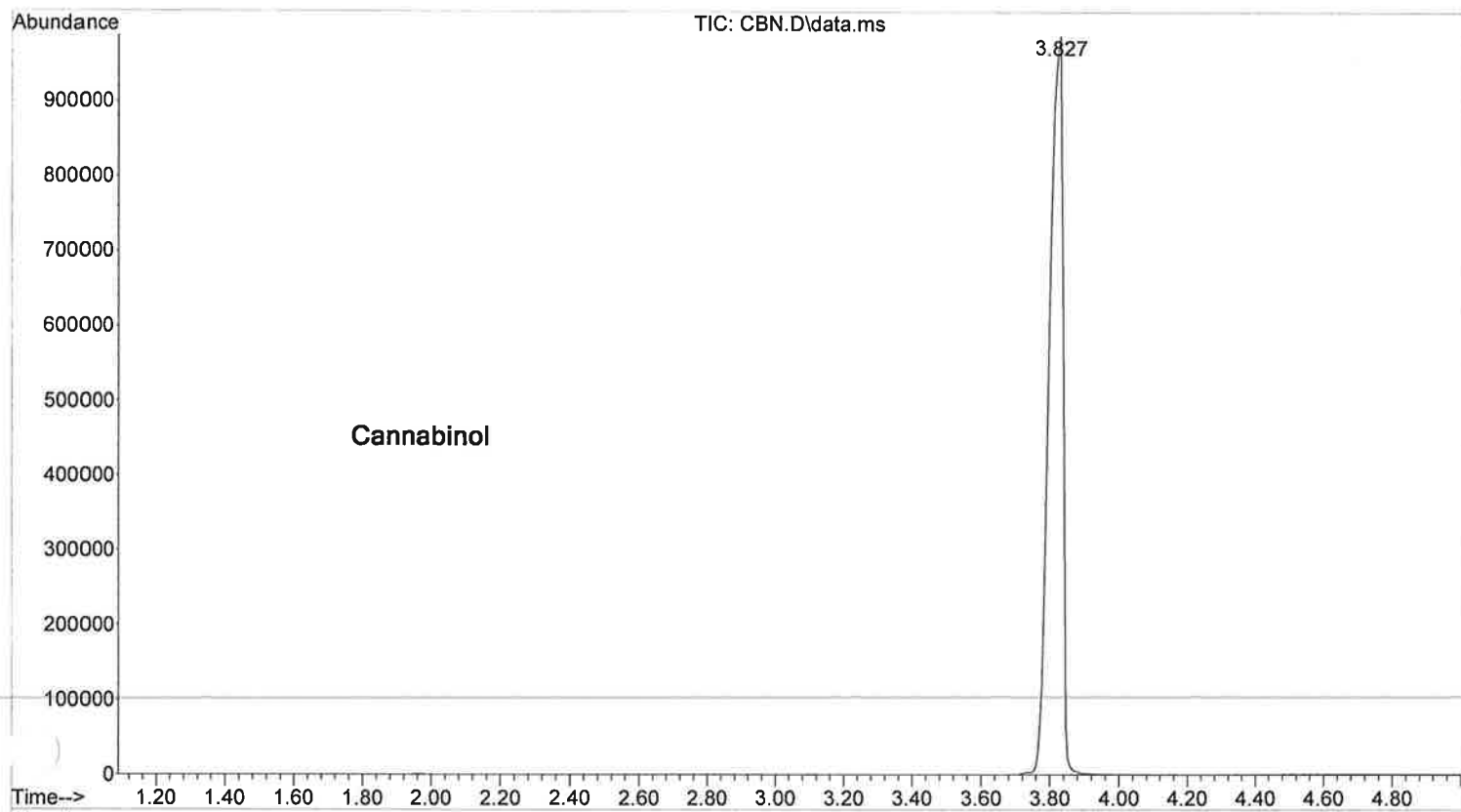
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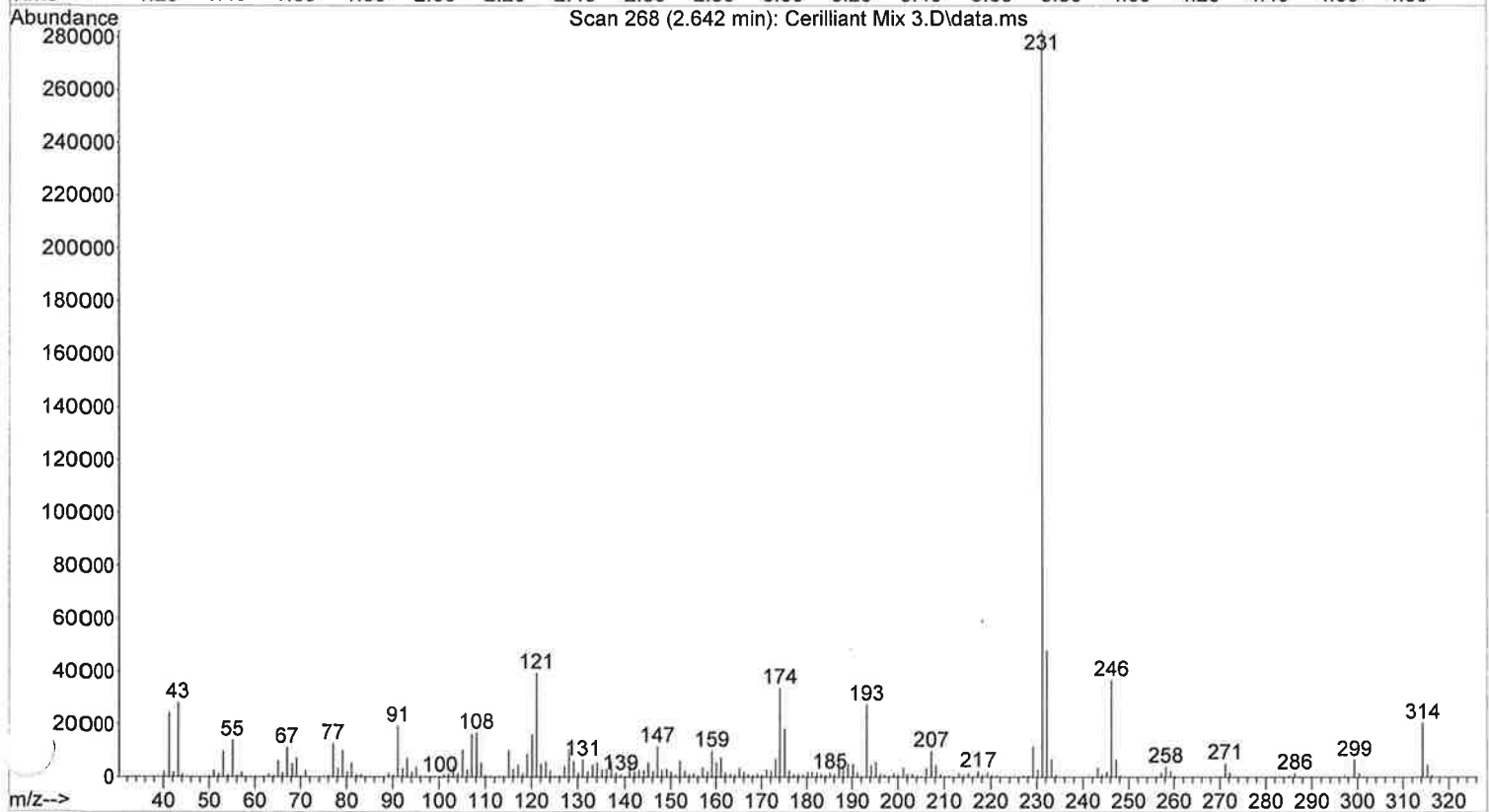
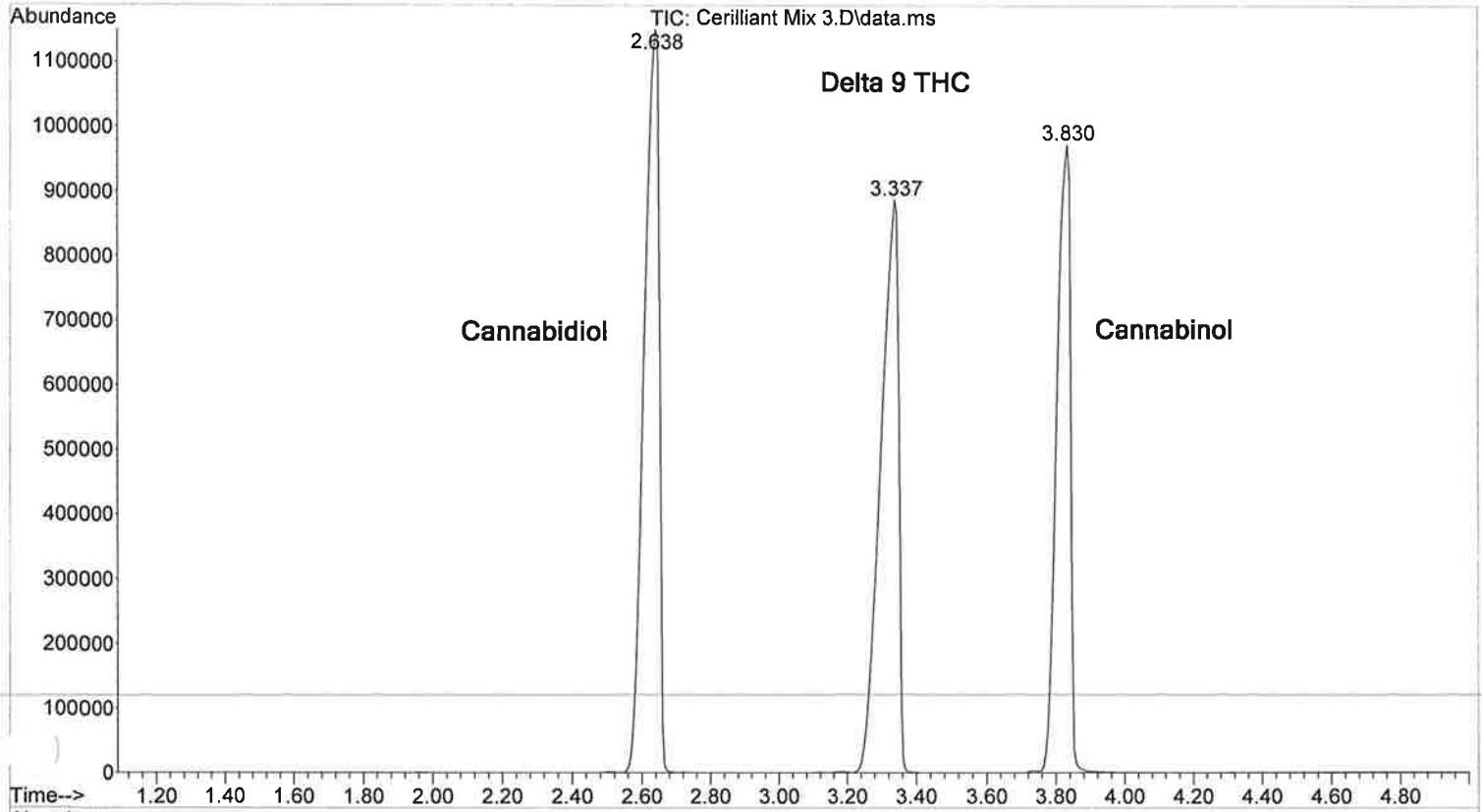
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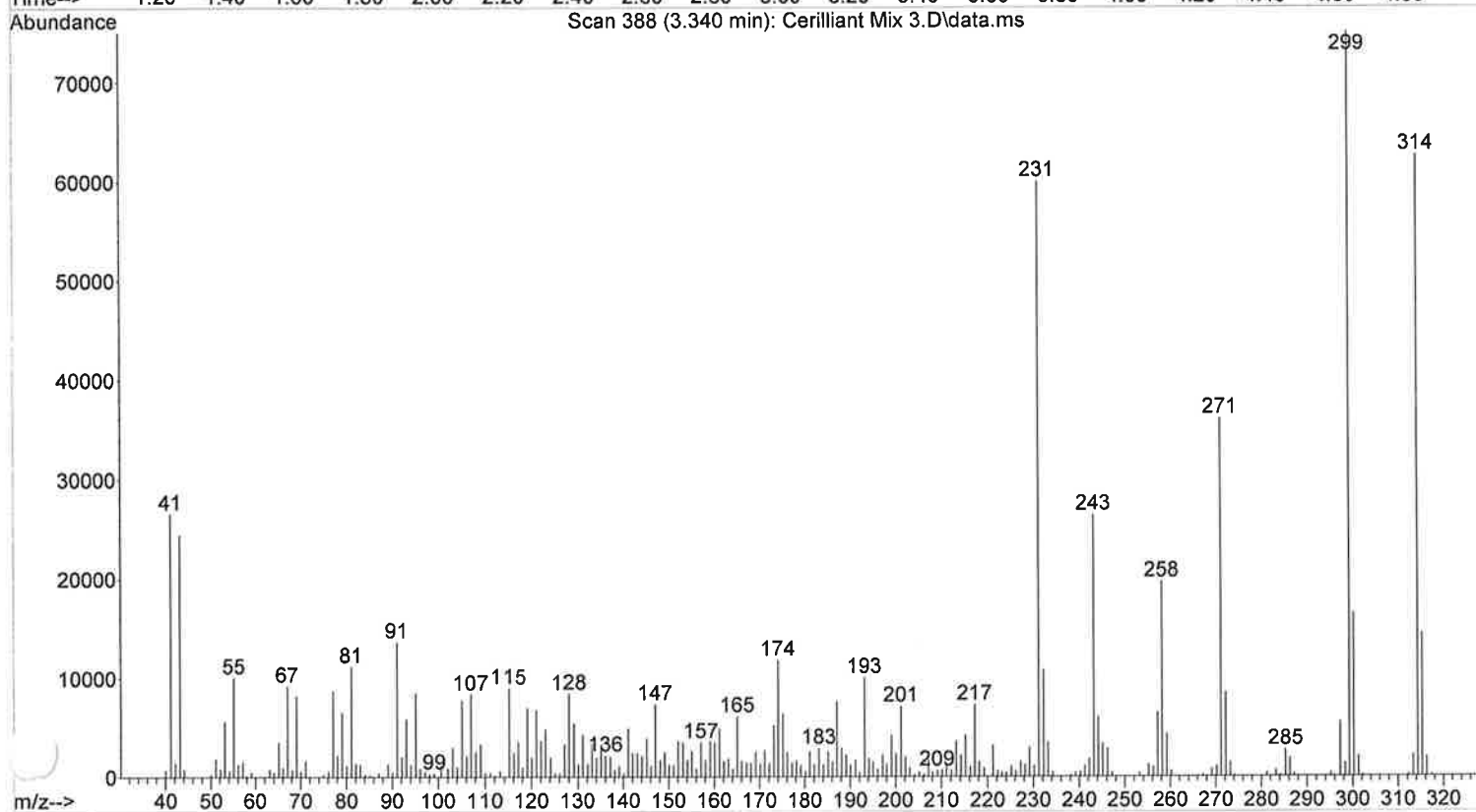
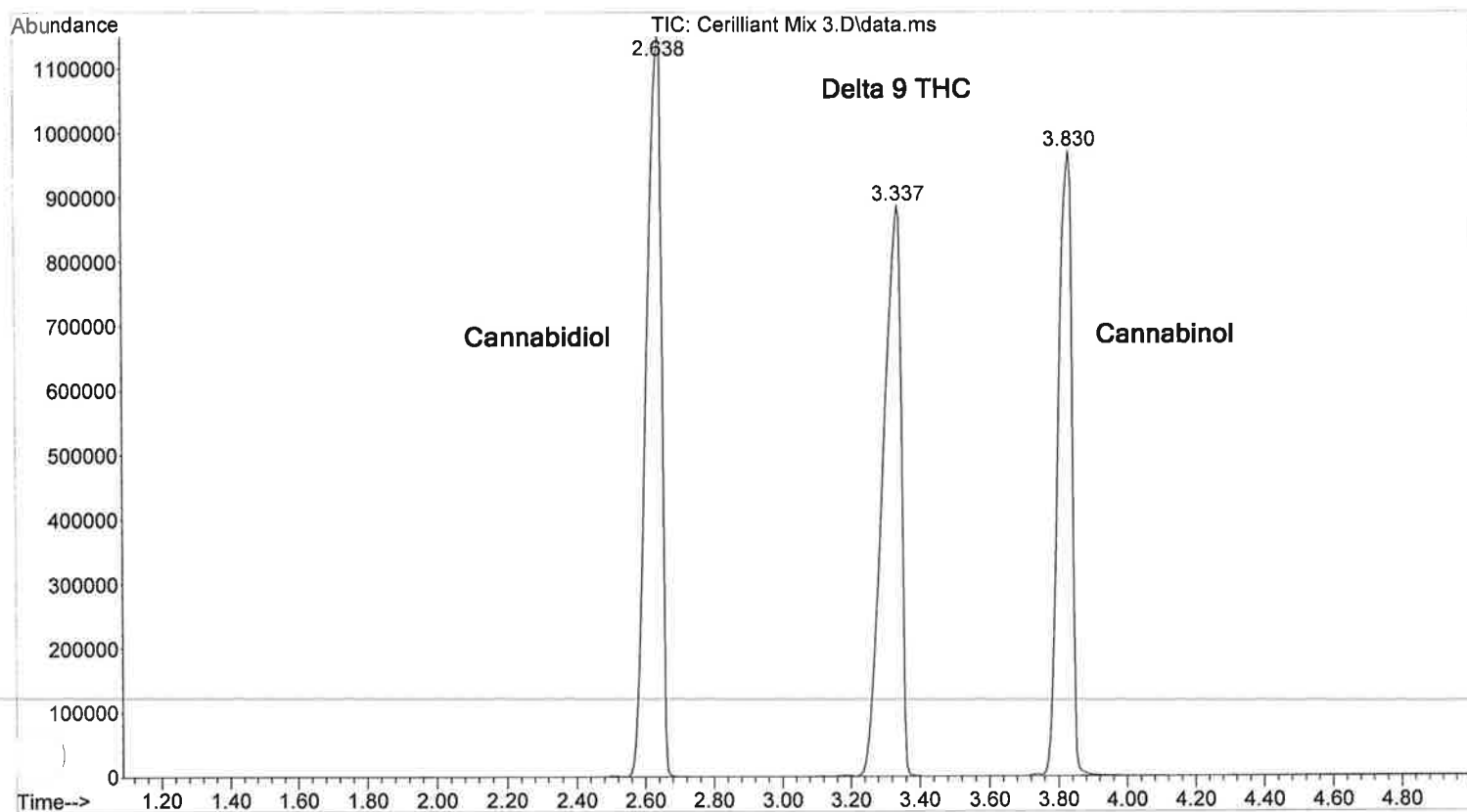
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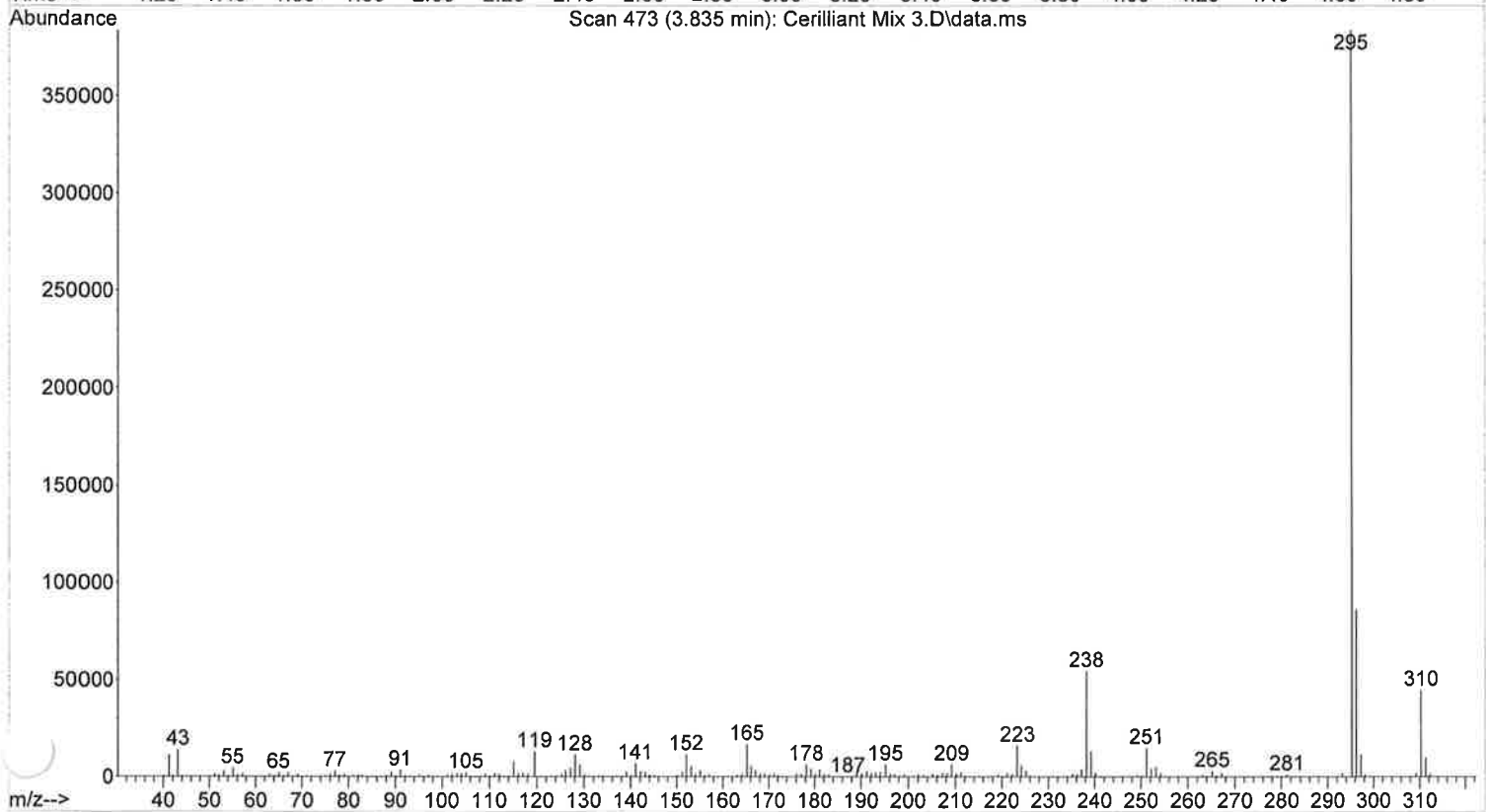
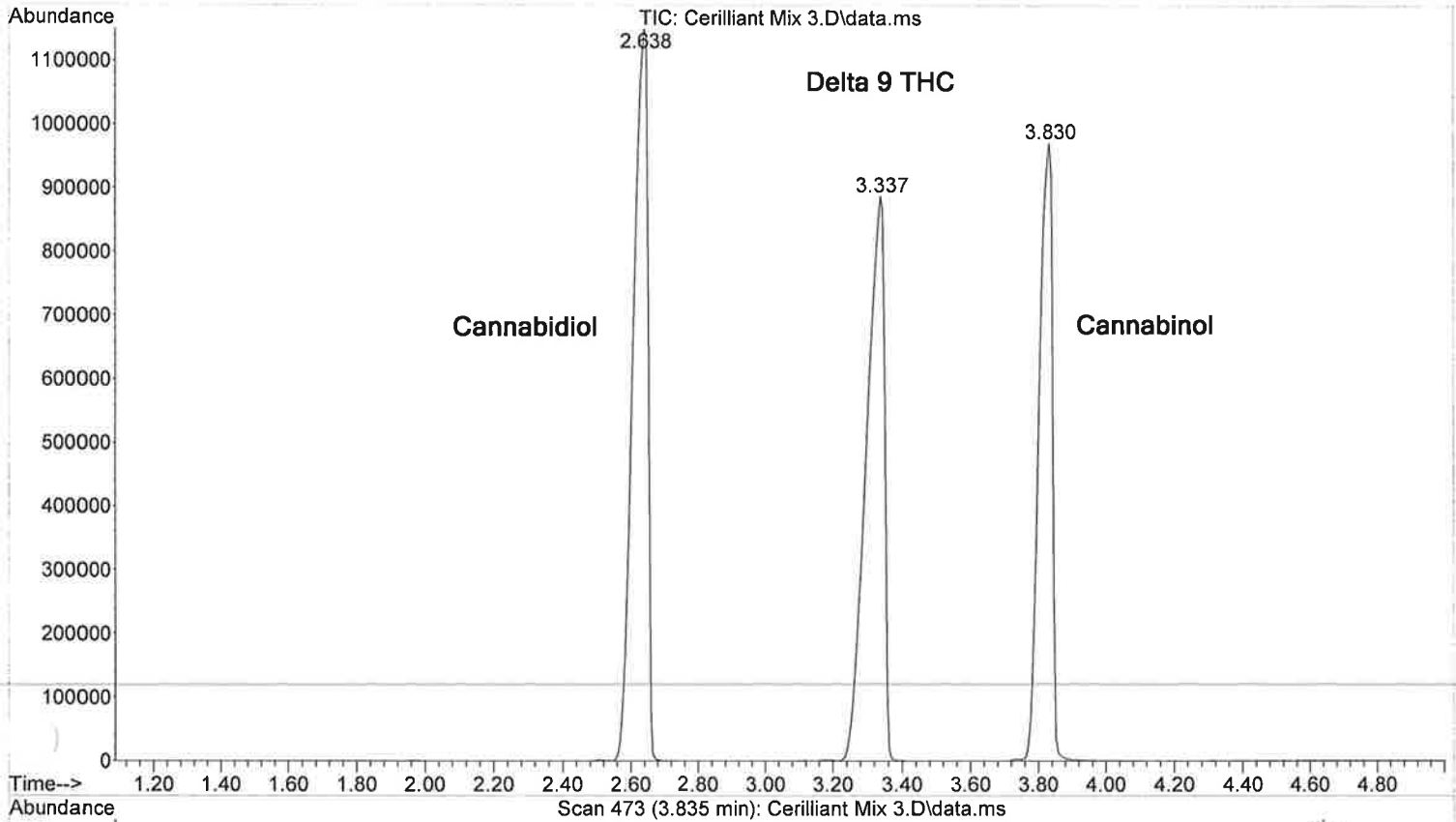
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