Cannabis Analysis III: Improved Separation of Terpenes and Terpenoids

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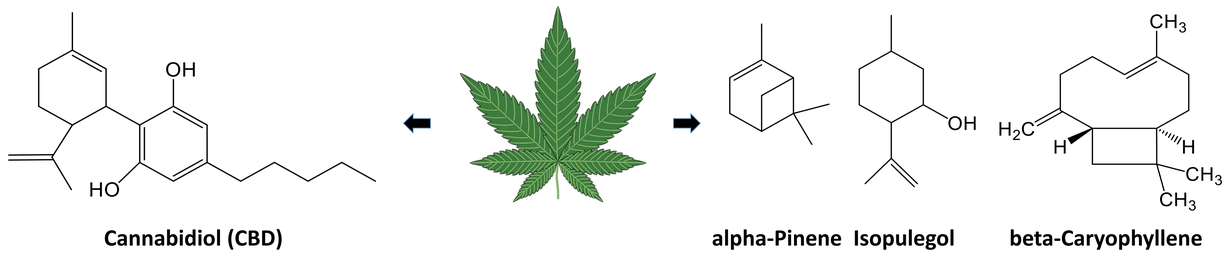
Introduction

The cannabis plant (*Cannabis Sativa L.)* produces a variety of compounds covering numerous chemical classes such as cannabinoids, terpenes, and terpenoids. Terpenes and terpenoids are responsible for the aroma characteristics and a variety of purported medical benefits. Terpenes are oligomers of isoprene units, whereas terpenoids are oxidized forms of terpenes. Analysis of these compounds is typically performed by Gas Chromatography (GC) via liquid injection or gas injection (Headspace). Due to the wide boiling point range of these compounds, operational cannabis laboratories most often perform the analysis using liquid injection. However, separation of the numerous and wide variety of compounds in the relatively short period of time needed for high throughput is challenging. We have determined that separation (selectivity) of terpenes and terpenoids commonly found in consumer products was improved by modifying GC column stationary phase chemistry. This improvement in selectivity will ultimately improve identification and quantitation of these compounds.

Cannabinoids | **Terpenes/Terpenoids**



Cannabidiol (CBD) | **alpha-Pinene** **Isopulegol** | **beta-Caryophyllene**

23 Terpenes/Terpenoids

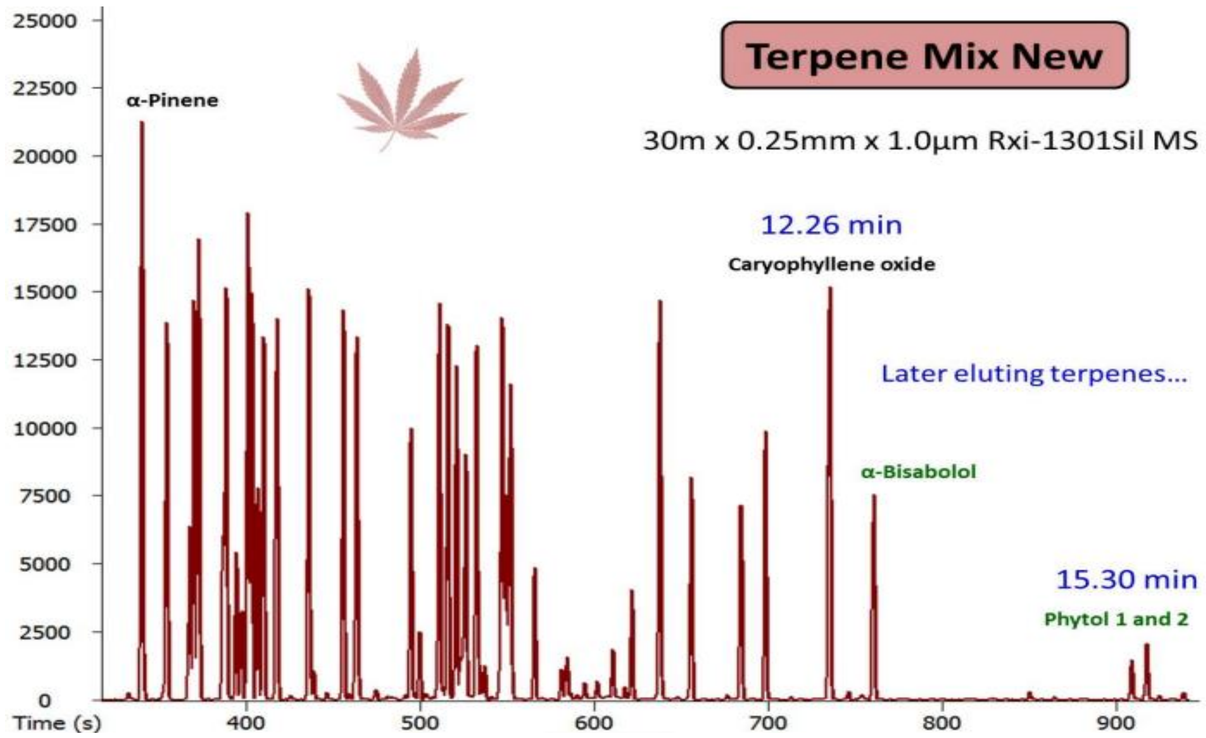
alpha-Pinene	alpha-Terpinene	gamma-Terpinene	beta-Caryophyllene	(-)-alpha-Bisabolol
Camphene	p-Cymene	Terpinolene	alpha-Humulene	Eucalyptol
(-)-beta-Pinene	d-Limonene	Linalool	cis-Nerolidol	(-)-Caryophyllene oxide
beta-Myrcene	cis-B-Ocimene	(-)-Isopulegol	trans-Nerolidol	
delta-3-carene	trans-B-Ocimene	Geraniol	(-)-Guaiol	

Experimental

Stock Terpene/Terpenoid Preparation: Standard solutions of terpenes were purchased at 1.0 mg/mL in isopropanol and diluted to 0.1 mg/mL with isopropanol.

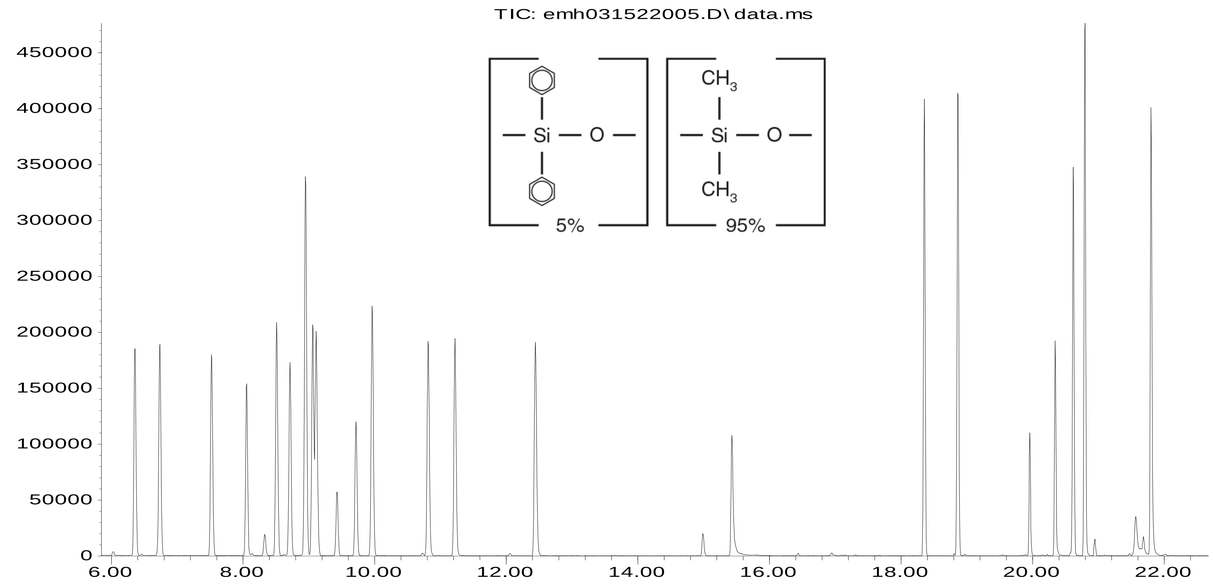
GC-MS Analysis: All analyses were performed on an Agilent 7890A/5975C GC-EI-MSD

Current Commercial Selectivity



Results

5% di-phenyl - 95% polymethylsiloxane



6% (phenyl/cyanopropyl) - 94% polymethylsiloxane

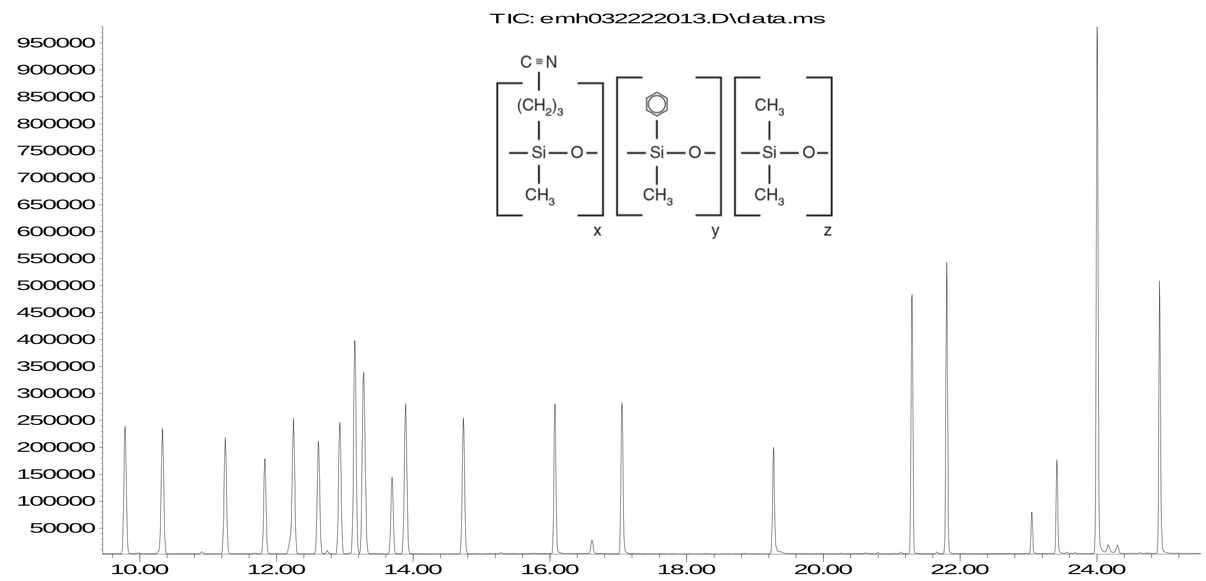


Figure 1: Chromatograms of 23 terpene/terpenoid mixture on low polarity (top) and mid-polarity (bottom) GC columns.

Conclusions/Future Work

- Separation of the terpene/terpenoid std mixture was improved using both column chemistries.
- 23 of 23 terpene/terpenoids were separated on the 5% di-phenyl - 95% polymethylsiloxane column (20 baseline separated).
- 21 of 23 terpene/terpenoids were separated on the 6% (di-phenyl/cyanopropyl) - 94% polymethylsiloxane column (19 baseline separated).
- Future work: identify each component with standards and optimize methods.

References

[1] Liktor-Busa E, Keresztes A, LaVigne J, Streicher J, Largent-Milnes T, Barker E Ed., Analgesic Potential of Terpenes Derived from Cannabis sativa; Pharmacological Reviews October 2021;73;4;1269-1297.

[2] Analysis of Terpene and Terpenoid Content in Cannabis Sativa Using Headspace with GC/MSD; Agilent Application Note, https://www.agilent.com/cs/library/applications/ application-terpenes-8890-gcms-headspace-5994-1497en-agilent.pdf

[3] Faster GC Analysis of Medical Cannabis Terpenes with Same 624Sil MS Selectivity; Restek Application Note, https://www.restek.com/en/chromablography/chromablography/faster-gc-analysis-of-medical-cannabis-terpenes-with-same-624sil-ms-selectivity/