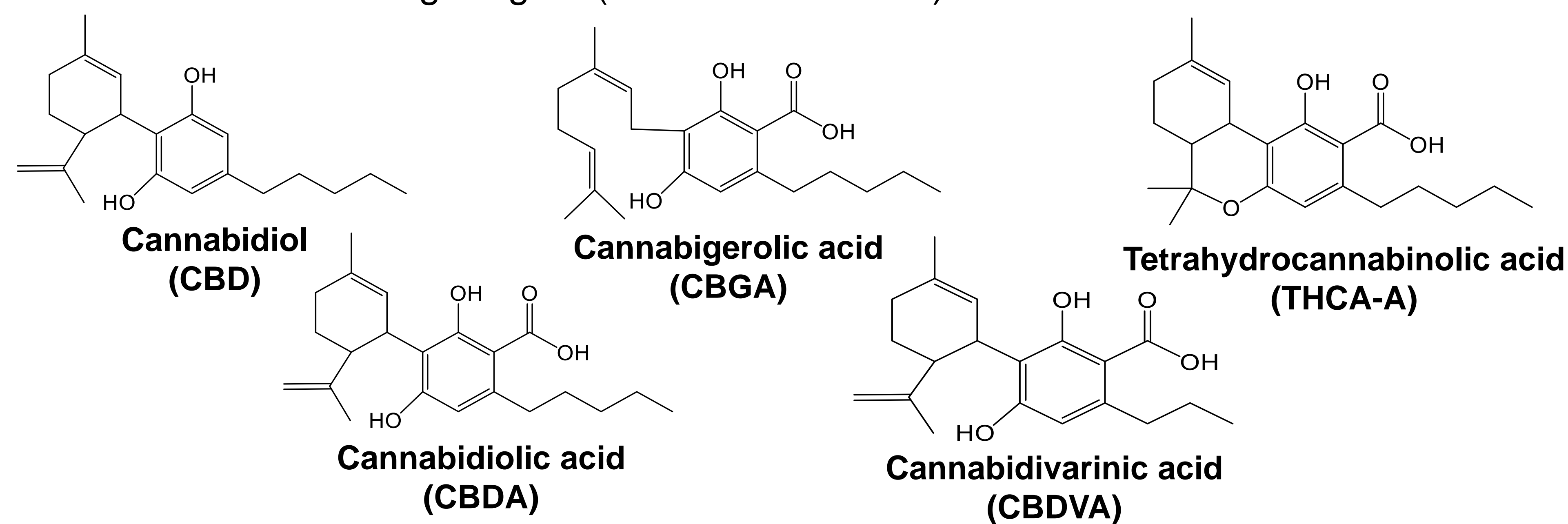


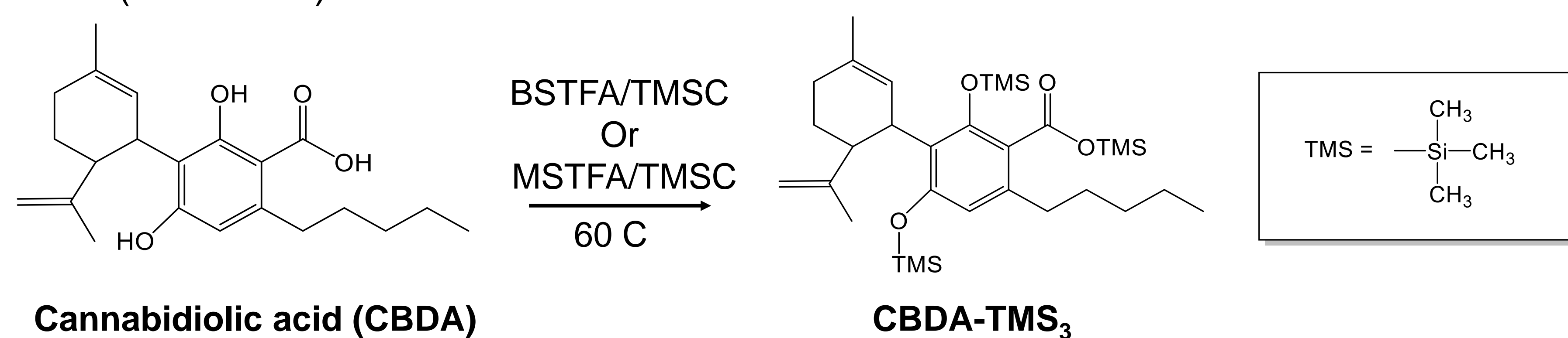
Introduction

The cannabis plant (*Cannabis Sativa L.*, aka *hemp*) produces a variety of compounds covering numerous chemical classes such as cannabinoids, terpenes, and terpenoids. Cannabinoids (acidic and neutral) are responsible for producing psychoactive and other pharmacological effects. In the first stage of this project, cannabinoid acids were studied due to their ability to bind to the spike protein of the COVID-19 virus and thus inhibit cell entry, replication, and infection. In this second phase, we expanded the number and types of consumer products studied. We applied our methodology to analyze three new consumer products in several matrices containing various combinations of CBD, CBDA, and CBGA. Since the cannabinoid acids are prone to decarboxylate at elevated temperatures and revert to their neutral counterparts (ex. CBDA to CBD), they are found formulated mainly into non-smokable consumer products. The elevated temperatures used in GC-EI-MS analysis necessitated the addition of protective functionality to active -OH groups via derivatization. This methodology proved to be effective at preventing decarboxylation and allowed the acids to survive the analysis. We also investigated the use of a new derivatizing reagent (MSTFA vs. BSTFA).



Analytical Approach

The neutrals and acids of interest were first derivatized with MSTFA/TMSC or BSTFA/TMCS which replaces active -OH groups with thermally stable -OTMS groups, then analyzed by GC-EI-MS (ex. CBDA).



Experimental

Sample Preparation and Analysis: DEA-exempt standard solutions of CBD, CBDA, CBGA, THCA-A, and CBDVA were purchased at 1.0 mg/mL in acetonitrile and diluted into stock solutions of 0.1 mg/mL with acetonitrile. Standards were then derivatized by addition of BSTFA (or MSTFA) containing 1% TMSC and heated for 30 minutes at 60°C. The cannabis consumer products (gummies and soft gels) were dissolved in water with sonication and heat, extracted with hexane, and evaporated under a dry air stream at 60°C. The resulting residue was dissolved in chloroform and derivatized as described above. Extraction of the powder-based cannabis product (isolate) was diluted in acetonitrile and derivatized using BSTFA with 1% TMSC. All analyses were performed on an Agilent 7890A/5975C GC-MSD.

Cannabis Consumer Products:



Results

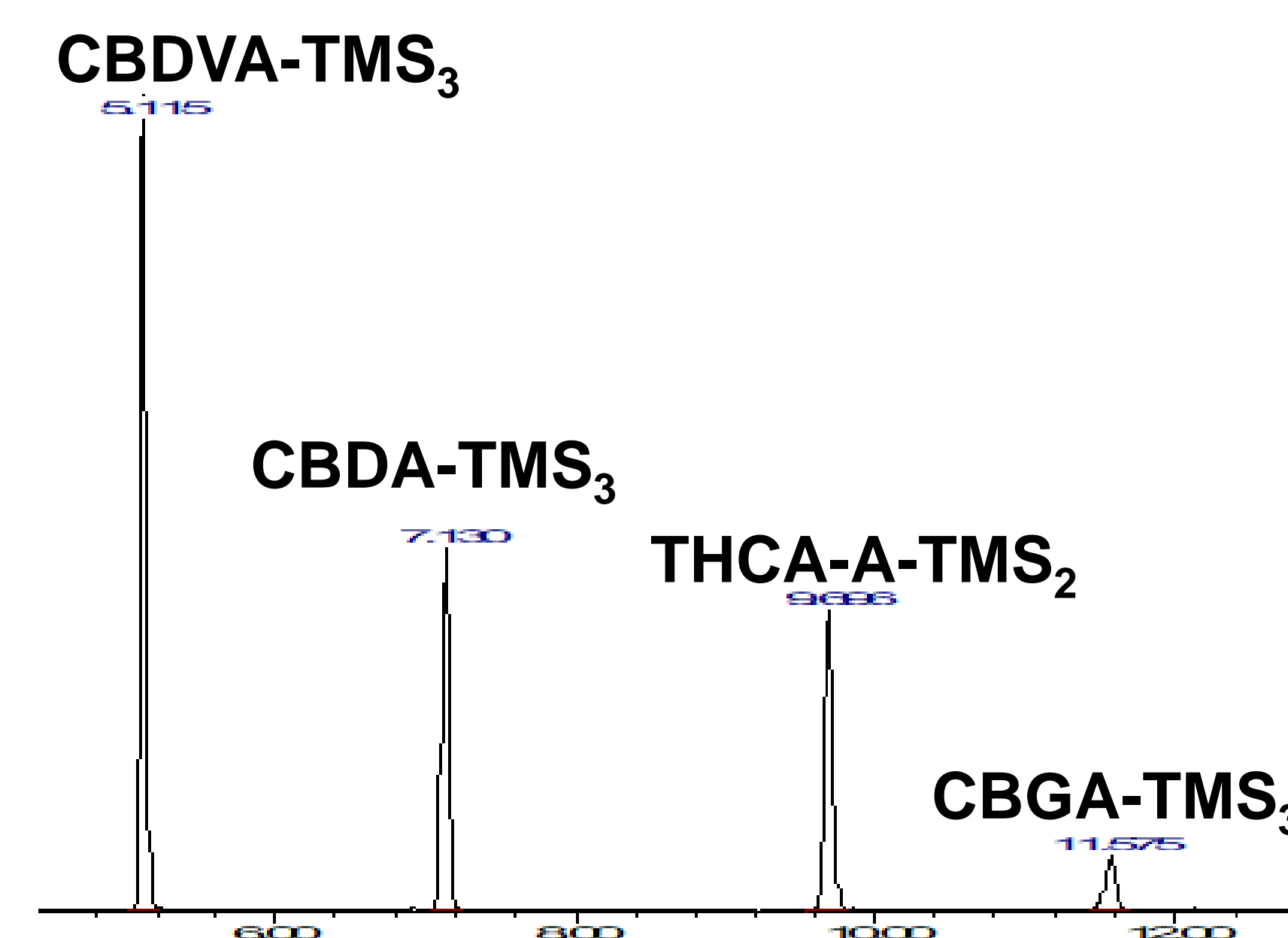


Figure 1: Gas chromatogram of four cannabinoid standards.

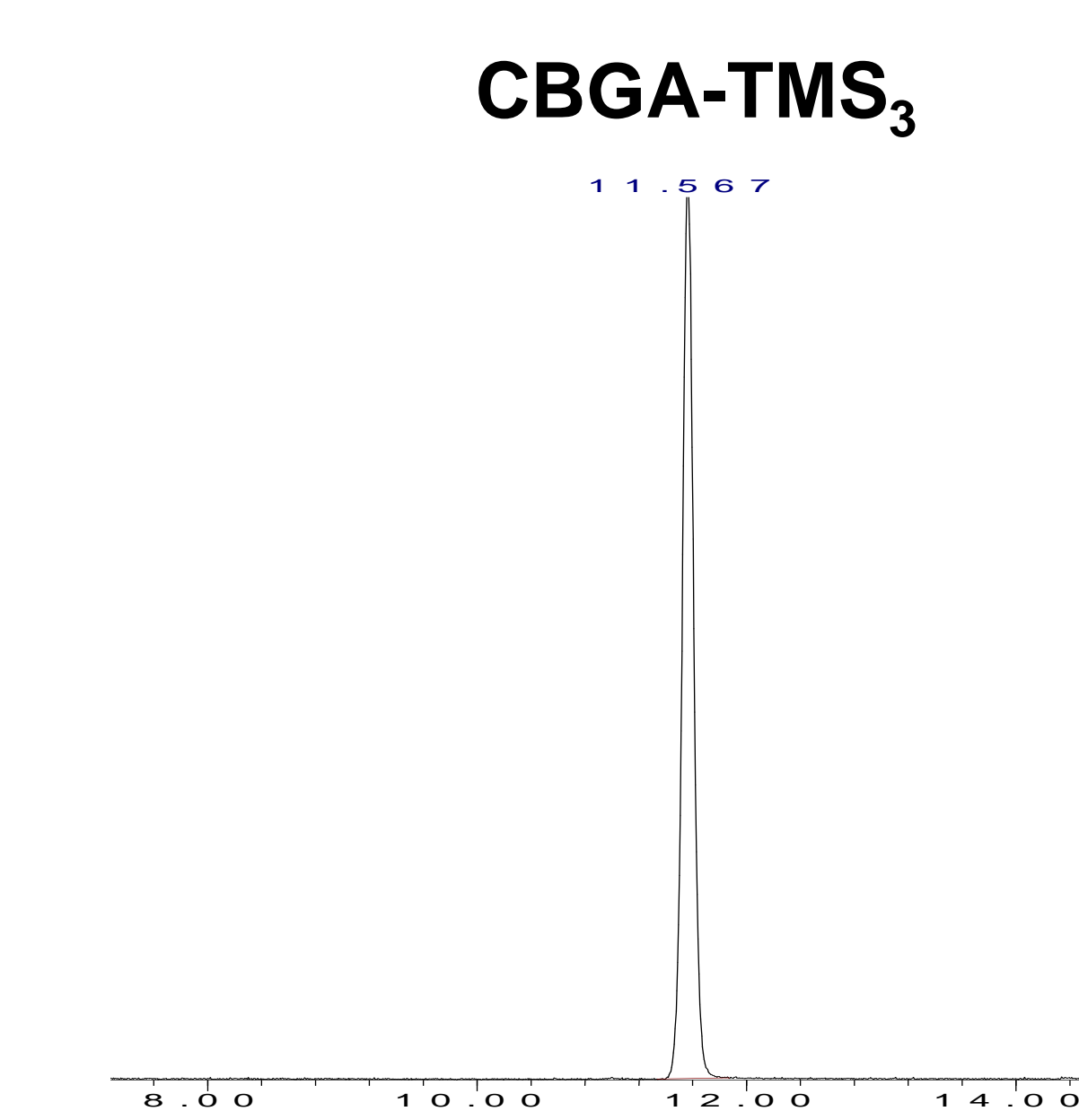


Figure 2: Gas chromatogram of CBGA in isolate product.

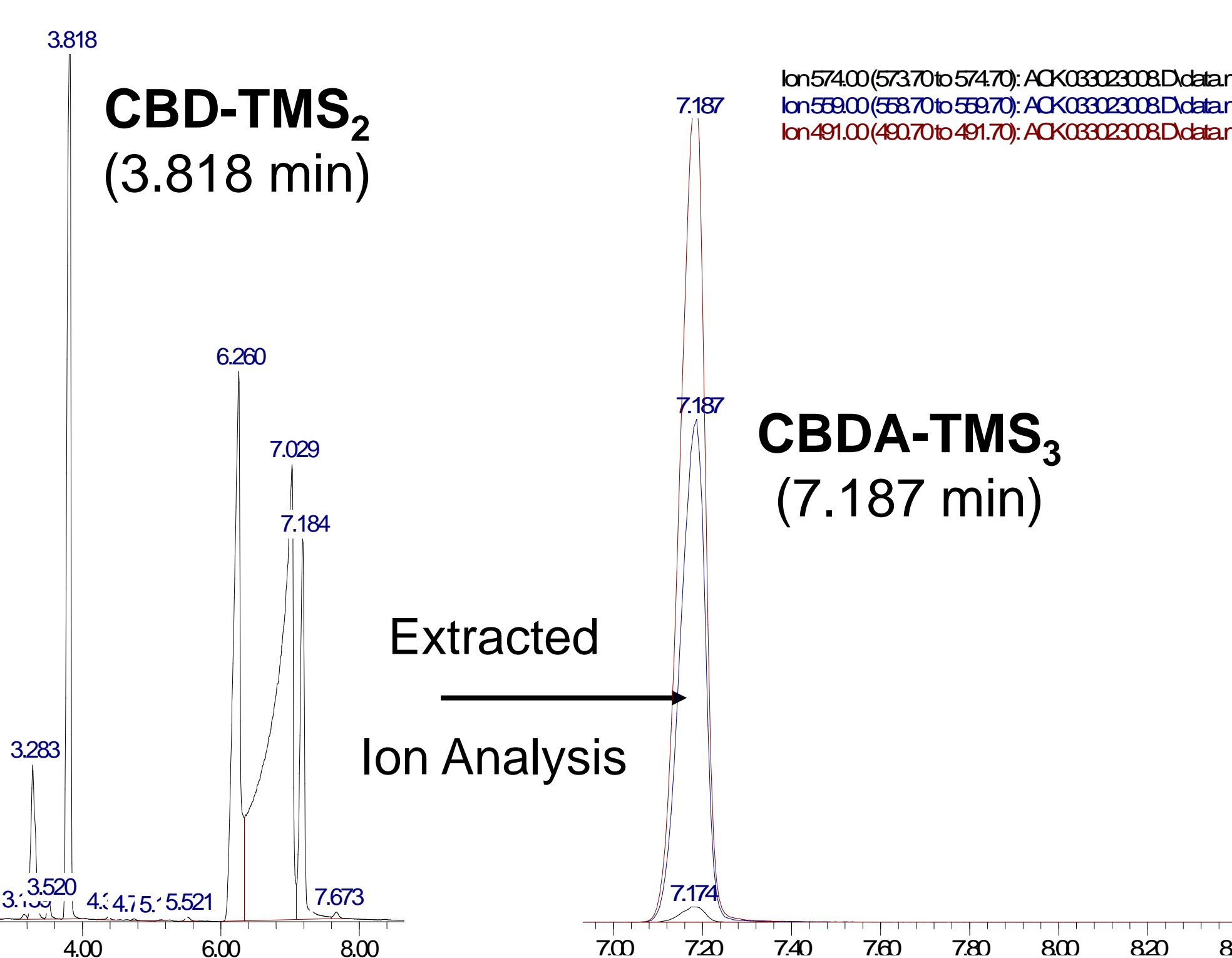


Figure 3: Gas chromatogram of CBD-TMS₂ and CBDA-TMS₃ in soft gel product. CBDA-TMS₃ detected using Extracted Ion Analysis, ions and normalized signal intensities calculated: 574 (1.83%), 559 (60.9%), and 491 (100%)

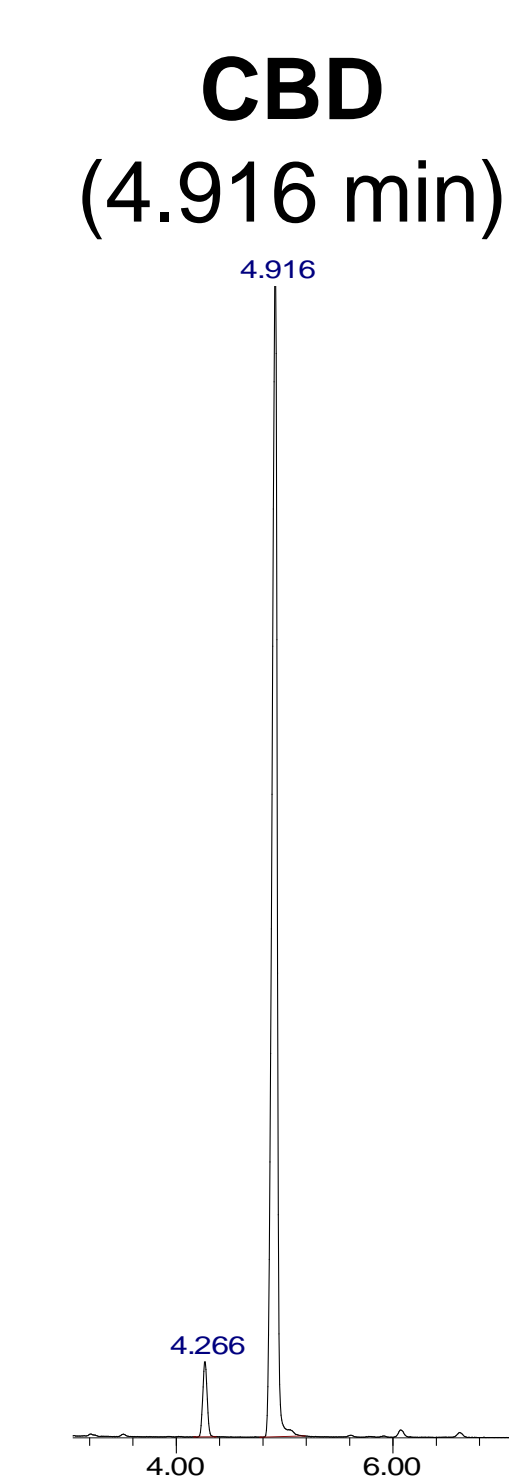


Figure 4: Gas chromatogram of CBD in gummy product (CBD-TMS₂ and CBDA-TMS₃ not detected).

Cannab.	MW (g/mol)	-TMS	-TMS MW (g/mol)	Ret. Time (min)	M+, m/z	M-15, m/z	Base ion, m/z
CBD	315	CBD-TMS ₂	458	3.788	458 (8.3%)	443 (5.9%)	390 (100%)
CBDVA	330	CBDVA-TMS ₃	546	5.115	546 (1.7%)	531 (58%)	463 (100%)
CBDA	358	CBDA-TMS ₃	574	7.13	574 (1.8%)	559 (63%)	491 (100%)
THCA-A	358	THCA-A-TMS ₂	502	9.696	502 (2.1%)	487 (100%)	487 (100%)
CBGA	360	CBGA-TMS ₃	576	11.575	576 (1.4%)	561 (100%)	561 (100%)

Table 1: Molecular weights, retention times, and mass spectral data for CBD and cannabinoid acids.

Conclusions/Future Work

- The choice of derivatizing reagent did not affect the GC-EI-MS data
- The method was successful in identifying CBD, CBDA, and CBGA in consumer products
- The retention times of cannabinoid acids in the consumer products was consistent with the retention times produced from the standard mixture.
- Future work will include testing a wider variety of consumer products containing different cannabinoids and developing a quantitative method for each analyte.

References

- [1] van Breemen R, Muchiri R, Bates T, Weinstein J, Leier H, Farley D, Tafesse F; Cannabinoids Block Cellular Entry of SARS-CoV-2 and the Emerging Variants; *J of Nat Prod*;2022 85;1;176-184.
- [2] Ciolino L, Ranieri T, Taylor A; Commercial cannabis consumer products part 1: GC-MS qualitative analysis of cannabis cannabinoids, *For. Sci. Int.*; 289:2018;429-437.
- [3] ElSohly M, Murphy T, Khan I, Walker L, Gula W; Analysis of Cannabidiol, Δ9-Tetrahydrocannabinol, and Their Acids in CBD Oil/Hemp Oil Products; *Medical Cannabis and Cannabinoids*;2020;3;1-13.