

# Cannabinoid Analysis

## Derivatization Principles

Joseph DiVerdi

CHEM 442 Chemistry of Hemp & Cannabis

Department of Chemistry

Colorado State University

Fort Collins, CO USA

April 2022

# Keep Our Eyes on the Goal

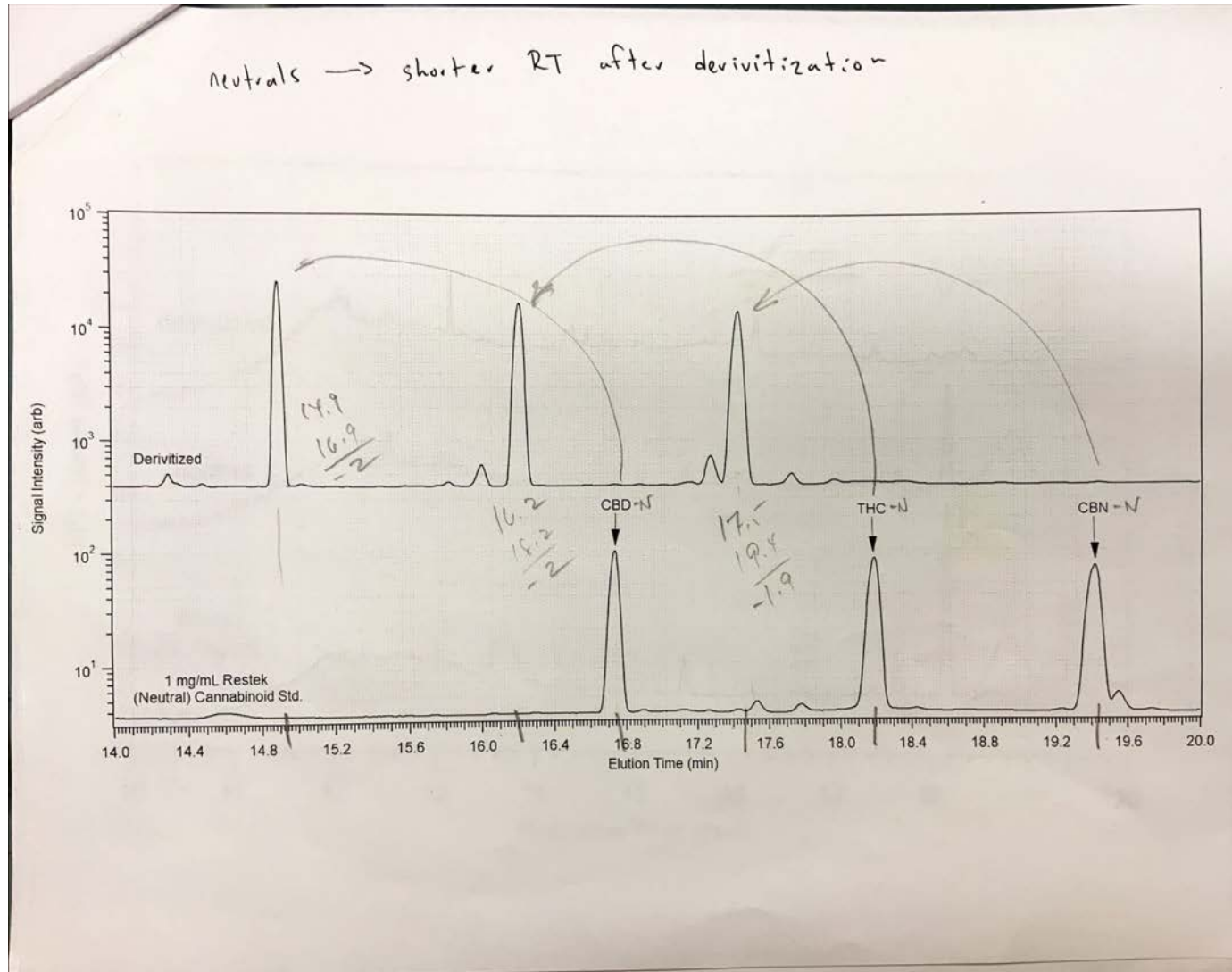
---

Colorado State University  
Fort Collins, CO

GC Retention Time (min)	CBD	THC	CBN
underivitized	16.75	18.20	19.42
derivitized N-form	14.92	16.20	17.48
derivitized A-form		19.20	

# There is no Doubt Here – Right?

Colorado State University  
Fort Collins, CO



# We Need to Fill in the Last Two Blanks

---

Colorado State University  
Fort Collins, CO

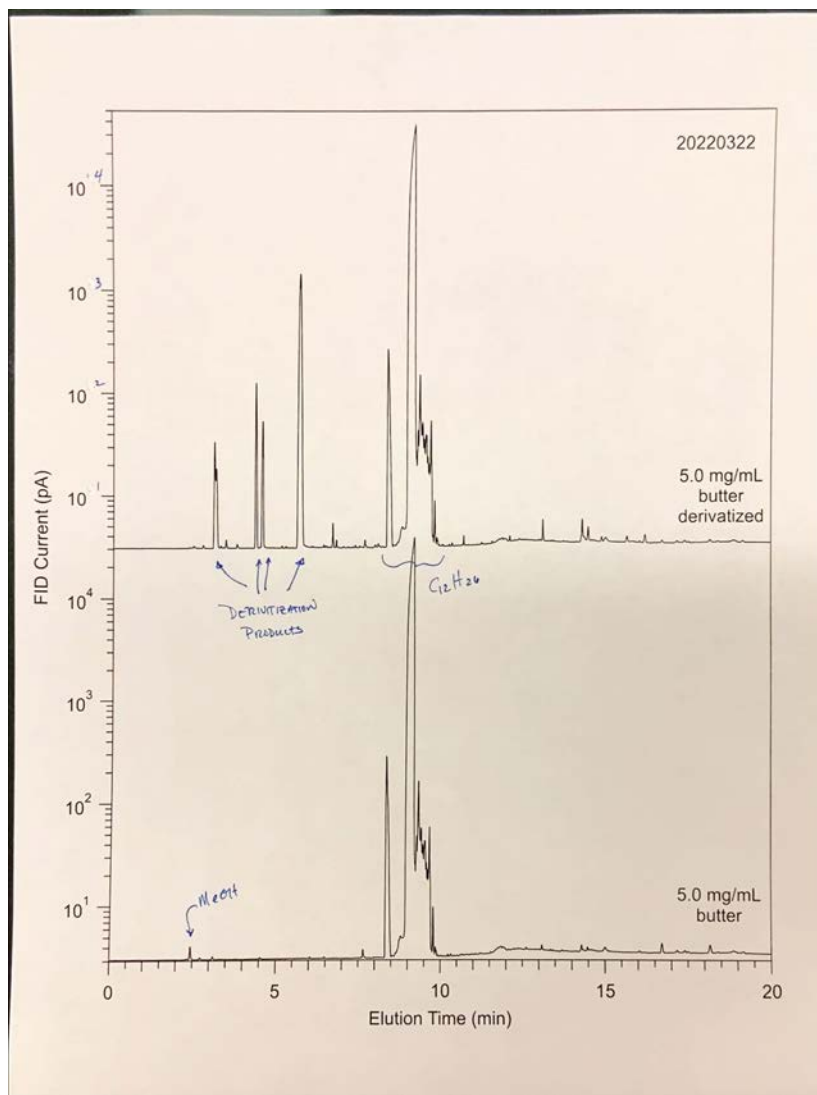
GC Retention Time (min)	CBD	THC	CBN
underivitized	16.75	18.20	19.42
derivitized N-form	14.92	16.20	17.48
derivitized A-form		19.20	

Can we accomplish this need  
with materials and tools (and  
brains) that we have at hand?

Let's take the problems one at a time:  
CBD-A first.

# Let's Orient Ourselves

Colorado State University  
Fort Collins, CO



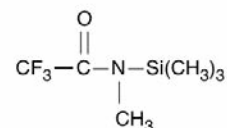
## Considerations for the use of MSTFA + 1% TMCS

MSTFA is an effective trimethylsilyl donor with donor strength approximately the same as BSA and BSTFA.<sup>1</sup> It reacts to replace labile hydrogens on a wide range of polar compounds with a -Si(CH<sub>3</sub>)<sub>3</sub> group. Therefore, it is used to prepare volatile and thermally stable derivatives for gas chromatography and mass spectrometry.

One of the particular advantages of MSTFA over other silylating reagents is the volatility of its byproduct, *N*-methyltrifluoroacetamide. MSTFA is the most volatile TMS-amide available and *N*-methyltrifluoroacetamide has an even lower retention time than MSTFA. TMS derivatives of small molecules can often be analyzed when made from MSTFA, because the by-products and the reagent usually elute with the solvent front. Silylating reagents containing the trifluoroacetyl moiety, such as MSTFA, act as cleaning agents for flame ionization detectors. When a large number of TMS derivatives is to be analyzed using FID, silicone deposits from the excess derivatizing reagent tend to form on the detector and reduce its sensitivity. This buildup is minimized when derivatizing with reagents based on trifluoroacetic acid because the silicone is volatilized as SiF<sub>4</sub>. Therefore, BSTFA and MSTFA are recommended over BSA for these applications.

MSTFA + 1% TMCS can be used at full strength or diluted with a suitable solvent such as pyridine. In most applications it is advisable to use an excess of the silylating reagent, and at least a two-to-one molar ratio of MSTFA per active hydrogen is recommended. Best results are obtained when the products of the silylation reaction are soluble in the final reaction mixture. Amides, many secondary amines and hindered hydroxyls will not be derivatized by MSTFA alone; however, when a catalyst such as TMCS is added, many of these compounds can be derivatized satisfactorily.

### MSTFA

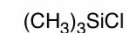


*N*-Methyl-*N*-trimethylsilyltrifluoroacetamide

MW 199.1

bp. 70°C/75 mm  $d_4^{20}$  1.11

### TMCS



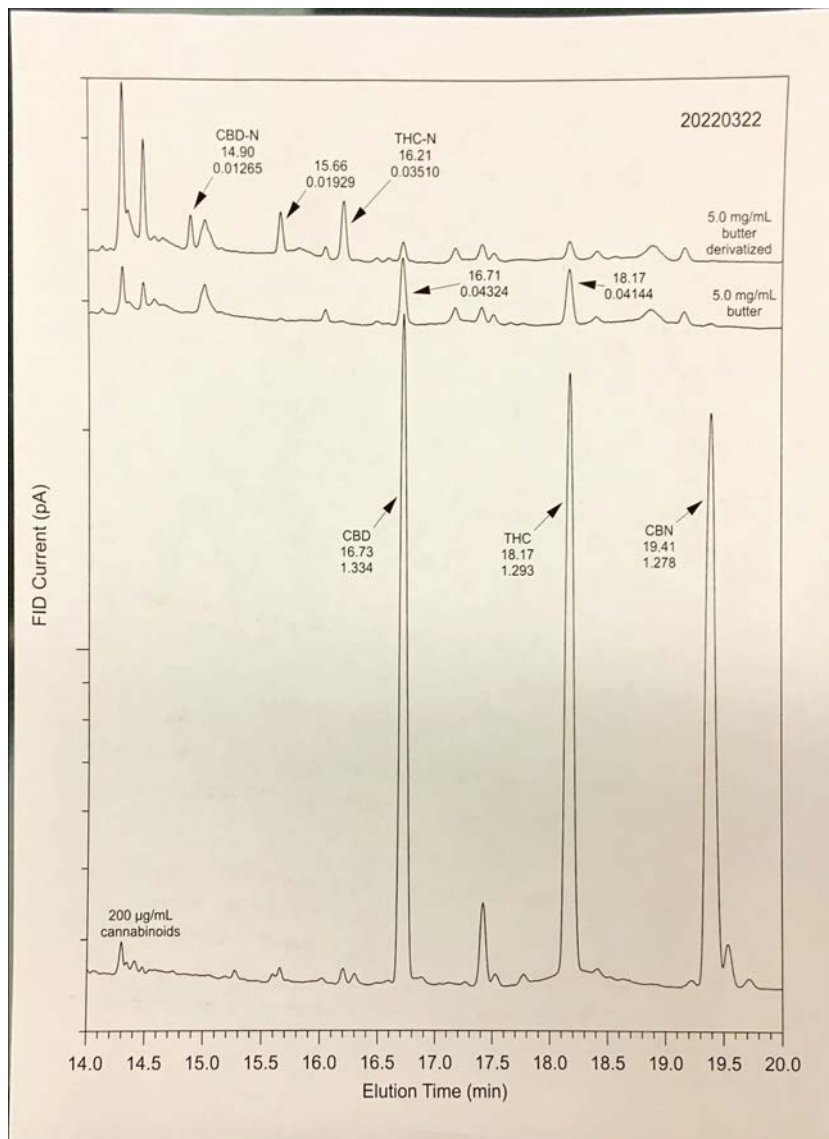
Trimethylchlorosilane

MW 108.64

bp. 57°C  $d_4^{20}$  0.858 mp -40°C

# Zoom into the Cannabinoid Region

Colorado State University  
Fort Collins, CO



Look for the “known peaks.

THC-N is present

And area makes sense

THC-A absent

Decarboxylation is complete

CBD-N is present

Other peaks are present

Could one of them be CBD-A?

# Alternate Hypotheses

---

Colorado State University  
Fort Collins, CO

1. Decarboxylation is complete for CBD (as it is for THC) and peak at 15.66 min is not CBD-A.
2. Decarboxylation is NOT complete for CBD and
  1. Either the peak at 15.66 min is CBD-A<sub>derivitized</sub>
  2. Or the peak is something else and is unknown

Recall also that:

THC<sub>underivitized</sub> : THC-A<sub>derivitized</sub> is +1.0 min

THC<sub>underivitized</sub> : THC-N<sub>derivitized</sub> is -2.0 min

CBD<sub>underivitized</sub> : CBD-N<sub>derivitized</sub> is -1.8 min



# Bottom Line

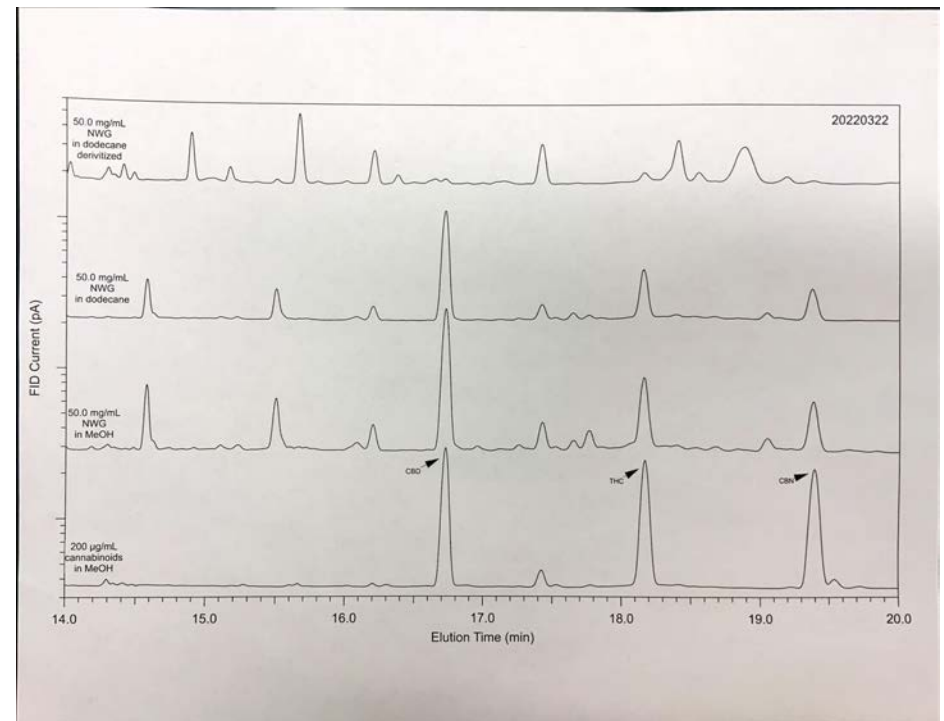
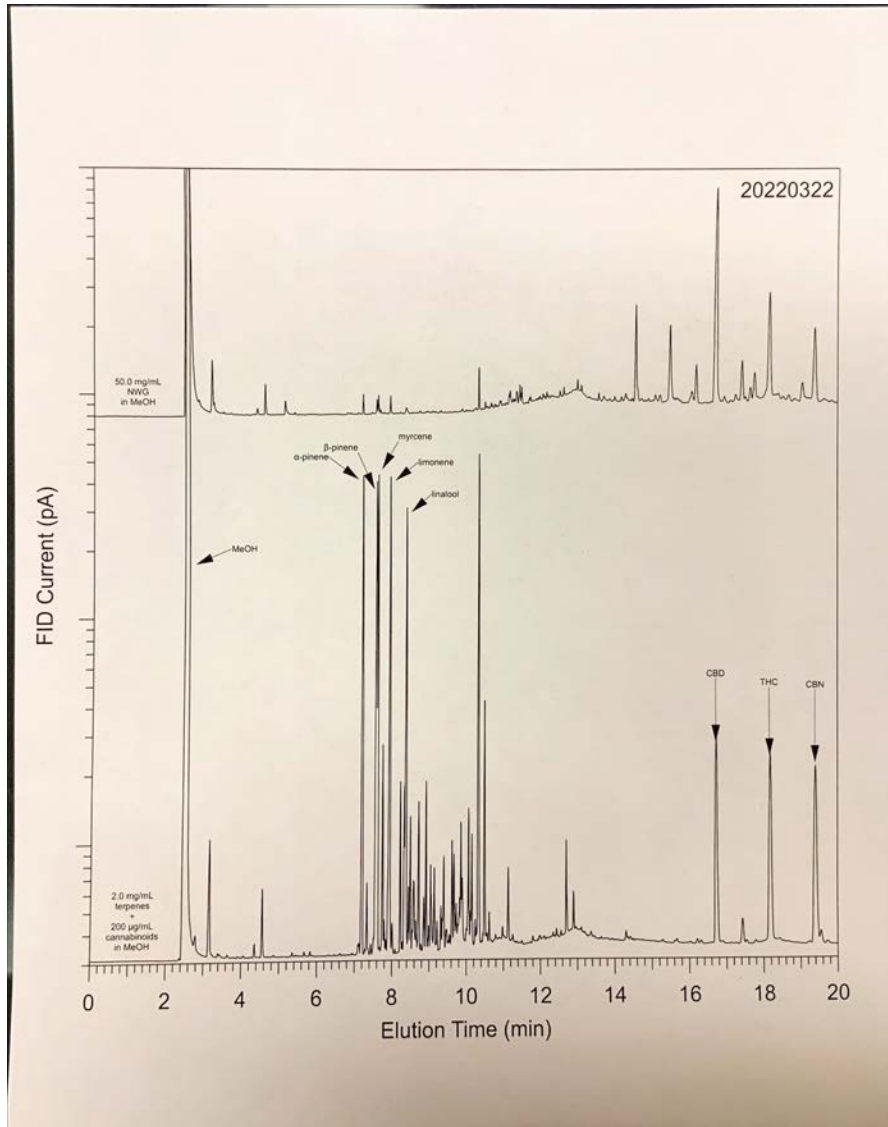
---

Colorado State University  
Fort Collins, CO

This sample (butter) is inconclusive in the determination of the elution time of CBD- $A_{\text{derivitized}}$

and we need to look elsewhere

# Another Possibility



You analyze it