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Rapid Method for Consistency and Concentration Reporting of Cannabidiol Using 1H-NMR and Computer-Assisted Chemical Software

Michael A. Fernando
Portland State University

Portland State University Honors College

Rapid Method for Consistency and Concentration Reporting of Cannabidiol Using ^{1}H-NMR and Computer-Assisted Chemical Software

by

Michael Fernando

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Thesis Advisor

Dr. Robert M. Strongin

Portland State University

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Rapid Method for Consistency and Concentration Reporting of Cannabidiol Using ^{1}H-NMR and Computer-Assisted Chemical Software

AvanceCore 400 MHz, TopSpin, CMC-Assist, PubChem, ChemDraw

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Michael Fernando, Robert Strongin

From Portland State University Department of Chemistry

Science and Research Center 1719 SW 10th Ave, Portland, Oregon, 97201

Abstract:

An integrated computational method was demonstrated with hemp-derived Cannabidiol for an assessment of its purity and concentration. The sample was structurally verified, high purity, and 2.98 $\frac{mmol}{L}$ in dissolved DMSO. The method presented is a general approach to assessing purity and concentration for any small organic molecule in CMC-Assist.

Introduction:

Proton nuclear magnetic resonance (^{1}H-NMR) is the benchmark method for elucidating chemical structures of small organic molecules. The instrument is nondestructive to samples requiring minimal preparation for acquisition, and no calibration curve for quantitation. Therefore, NMR has several key advantages over typical destructive methods used for analytical assays of purified small organic molecules. However, the traditional work-flow for processing data has been time intensive for complicated spectras. Assigning peaks, multiplicities, and generating descriptive statistics has restricted the use of NMR in field and lab quality assurance testing. Computational Assisted Chemical Softwares (CACS) provide a swift processing solution for NMR data of purified compounds and potential mixtures. The abundant hydrogen isotope and lesser abundant carbon-13 isotope are NMR active, i.e. the atomic nuclei undergo resonance in strong magnetic fields when pulsed with a radio frequency (RF)[1]. Fourier transform nuclear magnetic resonance spectroscopy (FT-NMRS) observes the pulsed nuclei's free induction decay (FID) over time. Signals are produced from the non-equilibrium nuclear spin of the magnetization vector precessing in a magnetic field.[1] A RF pulse near the Larmor frequency of NMR active atomic nuclei induces an oscillating voltage in a detection coil when the vector is non-zero in the xy plane[2]. The time-domain signal is then Fourier transformed to a frequency spectrum for analysis[2]. Peaks are separated based on their chemical shifts which are the resonant frequency of the atomic nucleus to a standard magnetic field . The integral peak areas represent the integer value of the hydrogens. Peak splitting, also known as multiplicity, arises from the nucleus being "split" by neighboring hydrogens found in different local chemical or magnetic environments. Structural analysis takes on even greater complexities when considering enantiomers requiring 2D experiments containing 2 atomic nuclei. Multiple atomic spectra are combined

for full detailed structural analysis of heterogeneous compounds including proteins and nucleic acids. Fortunately, most of the relevant chemical structure features of a small organic molecule can be identified from its hydrogen spectra.

ChemDraw is a chemical structure generating software. It can populate structures based on the International Union of Physical and Applied Chemistry (IUPAC) systematic naming. Names can be found on the PubChem website. Common or generic names are popular search keys to identify target molecules. The integration of these two applications allows for any molecule of interest to be generated for publication or animation without needing to recreate the structure.

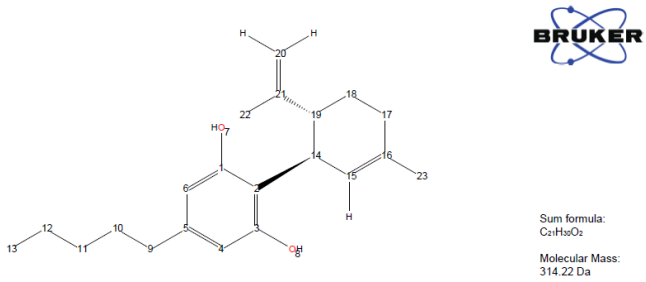


Figure:1 Cannabidiol Structure Imported from ChemDraw into CMC-Assist for Processing

Cannabidiol (CBD) is a major component of Cannabis *Sativa* L. CBD's IUPAC name is 2-[(1*R*,6*R*)-3-methyl-6-prop-1-en-2-yl cyclohex-2-en-1-yl]-5-pentylbenzene-1,3-diol. This name provides all the key information as to the molecule's primary moieties, functional groups, and stereochemistry. There are three 3 distinct moieties in CBD: phenol, limonene, and n-pentane like motifs. Limonene is a monoterpene existing as two entainomers i.e. and has stereochemistry (handedness). Phenols are benzene derivatives containing hydroxyl functional groups (OH) found in many organic molecules. n-Pentane is a straight-chain alkane commonly produced from processing fossil fuels. The hydrogen atoms along these carbon backbone motifs disperse throughout the captured proton nuclear magnetic resonance spectrum. Partitioning the molecule into these moieties helps for multiplet analysis.

CMC-Assist verifies chemical structures and generates full multiplet analysis reports. The program provides concentration, purity, and potency values in PDF for publication. The goal of this study was to create a control spectra of Cannabidiol for comparison in future studies of cannabinoids undergoing thermal degradation by smoking or dabbing. The control spectra will be used to elucidate toxicants formed by subtracting the known peaks of CBD in the degraded samples. The use of PubChem and ChemDraw allows for the rapid generation of any structure in CMC-assist.

Experimental Procedures:

Either CBD or Cannabidiol is searched for in PubChem for the desired molecular compound's entire profile. From PubChem, the IUPAC, SMILES, and various other names can be extracted, but only the IUPAC was needed for this procedure. Each of these names acts as a string of text used by various chemical software providers to generate chemical structures in 2D and 3D for publications and animations. After generating the desired structure in ChemDraw, a .Molfile, the molecular source code unique to the target compound is saved. Bruker's CMC-Assist imports this .mol file to generate a structure used to assess sample consistency and concentration. The software algorithm has in its backend predicted all of the chemical shifts of each Hydrogen and Carbon nuclei and to reference tetramethylsilane (TMS) located at 0 ppm.

Results and Discussion:

Two reference spectrums of Ibuprofen and Caffeine were analyzed against chemical standards and both reports generated very high purity. With the method verified, a commercial sample of CBD was given the same treatment and the sample concentration was found to be very high purity, consistency:ok, and 2.98 mmol/L. Multiplet interpretations for all major signals in the spectrum were found and the spectrum and structure were in agreement. Purified substances provide for computable verifiable NMR structures. CBD .mol files were imported from ChemDraw by systematic molecular naming extracted from PubChem. The ChemDraw structure was saved as a .mol file. Uploading the .mol into CMC-Assist or TopSpin calculated the chemical shift of all the hydrogen nuclei. After NMR experimental data was obtained the two spectra were processed against one another for consistency and concentration.

Conclusion:

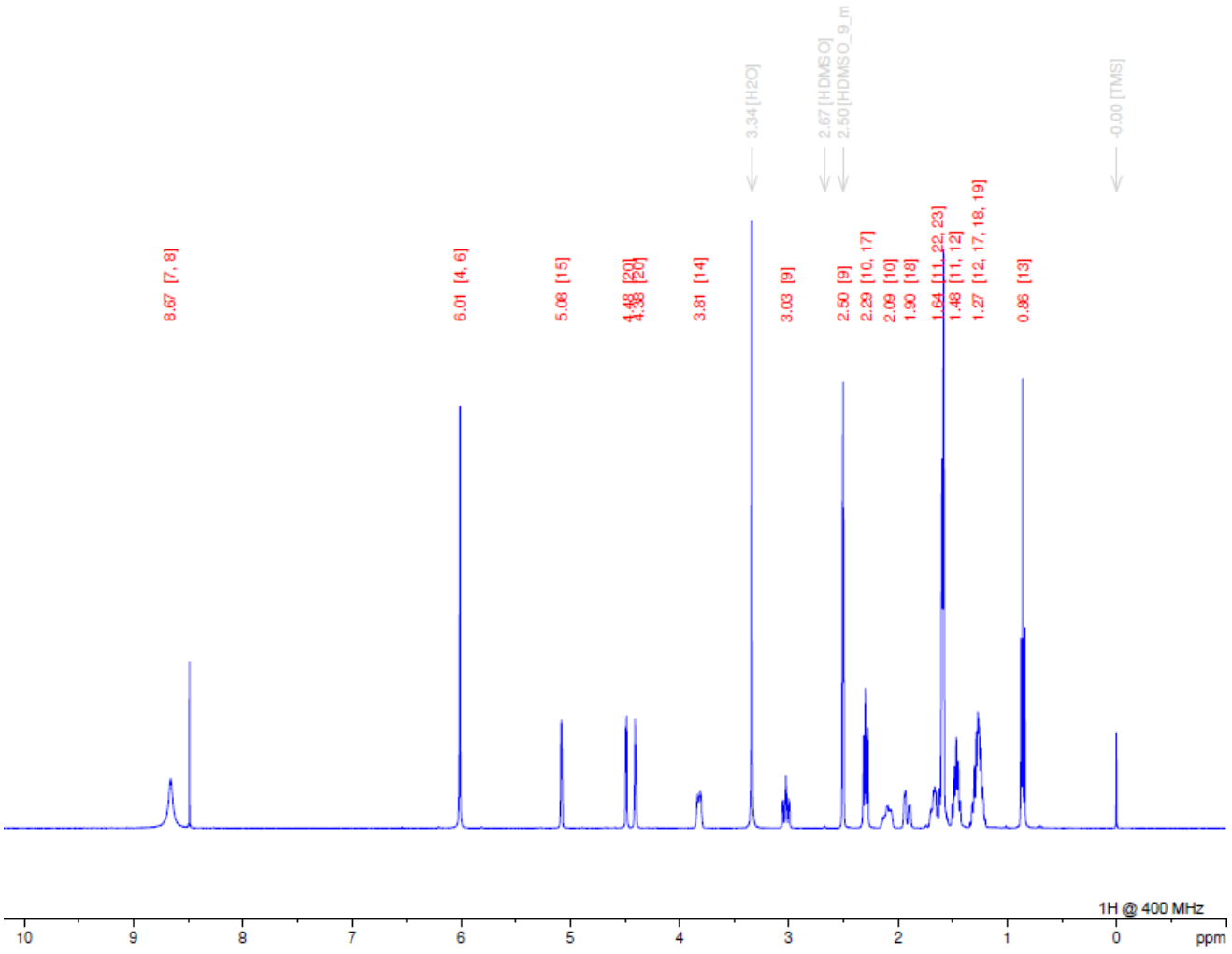
Cannabidiol's popularity has grown in recent years. Quicker newer solutions now exist to assess the purity, potency, and qNMR of mixtures. The method presented is being used to analyze the toxicants formed when CBD when smoked and vaped. It may also be deployed to monitor the purity of narcotics being confiscated by drug users and dealers. The purity of narcotics diminishes from its point of manufacture. This method would help to identify any suspected narcotic quickly and determine how pure the substance is. Deviations from expected spectra would indicate that contamination, byproducts, or alterations have occurred. Finding these anomalies could help to identify drug trafficking suspects by signature cuts, or residual byproducts from clandestine lab production wanted by law enforcement and drug task agencies.

References:

[1] Joseph P. Hornak. "The Basics of MRI". Rochester Institute of Technology. Chapter 4: NMR SPECTROSCOPY.

[2] Duer, Melinda J. Introduction to Solid-State NMR Spectroscopy. Blackwell Publishing, 2004, p. 43-58.

Figure 3: NMR Multiplet Analysis



*Numbers correspond to Figure 1