



Steep Hill™

Near Infrared (NIR) Spectroscopy for Potency Analysis of Cannabis

NIR and the Quality of Goods

Regulatory guidelines exist for testing the quality of food products, oils, and medicines. Quality and labeling of goods is regulated to assure that consumers purchasing those goods are getting what is on the label, or that the goods aren't spoiled. This quality assurance can be approached using conventional wet methods or by more modern rapid testing or "process analytical" testing methods. While conventional wet methods, including chromatography, are very versatile at testing major, minor and trace constituents, process methods can offer rapid, solvent-less measurement with good accuracy on major constituents.

Near infrared spectroscopy has, over the last two decades, become a preferred method in cases where materials are heterogeneous and require multiple samples, or in cases where blending requires a more rapid assessment to control blend content on the fly. Industries that use near infrared extensively for quality monitoring include finished product testing of oil and gas, grains, cheese and meat, and pharmaceuticals. The most prominent advantages of near infrared over chromatography include non-destructive and solvent-less testing, rapid turnaround time, much lower capital and maintenance costs, and a notably lower level of training required by the operator. For the testing of bulk components, NIR can be very useful on heterogeneous materials where several different samples can be used to determine bulk contents as in the case of protein, fat and water in grains.

Why NIR and Cannabis Potency?

Near infrared and cannabis seem to be a natural fit since the method has been very successful in assessing bulk contents of other agricultural products where it is currently used extensively. Since cannabis flowers are another agricultural product, NIR is a natural choice for application development of a cannabis potency analyzer. Cannabis is typically 5-30% cannabinoids (mainly THC and CBD) and 5-25% water. Cannabinoid content can vary by over 5% (e.g. 13-18%) on a single plant, and even more if grown indoor. This means that wet analytical methods, which are generally more accurate on a single sample, lose their accuracy advantage on a cost-per-analysis basis since more frequent testing is advised for batches likely to have such variability. Multiple NIR measurements are much more economical to estimate or correct for heterogeneity. Additionally, the NIR analysis uses no polluting solvents to dissolve samples and has a speed advantage of at least 5-10 times over wet methods at similar accuracy on heterogeneous materials.

The Making of an NIR-based Cannabis Analyzer

The use of an NIR-based analyzer system is notably simpler compared with wet methods like chromatography. Developing an NIR calibration does require knowledge and experience in the multivariate modeling with these analyzers. Model development is achieved by collecting NIR spectral measurements on a wide array of samples and wet method analysis for comparison on those same samples. Once an adequate number of samples is collected, a data processing of these “training” samples allows the creation of a mathematical model that relates the NIR spectral signatures with the concentrations determined by wet methods (HPLC). For example, spectra are collected on cannabis samples of various cannabis strains and Cannabinoid concentrations. Next a model is computed that relates the spectrum to the constituent concentrations. Finally, the model is validated using the regression solution and new samples not used in calibration to predict potency. NIR potency vs the wet method is then assessed to validate the model. Future samples can be measured on the NIR and cannabinoids for those new samples can be predicted. While the synopsis above sounds relatively simple, success depends on gathering enough of the appropriate data and understanding the use of the mathematical methods used in model building. The discipline of “Chemometrics,” also called “Process Analytical Technology,” is focused on the developing of these models. A good understanding of the mathematical method, physics of the spectrometer measurement, chemistry of the samples, and preferably experience in practice is desired. But once the model is built, a person with a high school education can be trained to operate the analyzer.

The Steep Hill Solution: QuantaCann2™

The QuantaCann2™ cannabis analyzer uses chemometric modeling of NIR spectroscopy and “training” data using wet chemistry (HPLC) to estimate the content of cured cannabis flowers in percent by weight content of four cannabinoids: THCA, $\Delta 9$ -THC, CBDA, and CBD. These are, by far, the most prevalent cannabinoids found in commercial cannabis. (The next highest in content would be CBGA and CBG.) Once an instrument is “calibrated” using the training data, many additional samples (samples not used in the training data calibration) are subsequently run by both NIR using the QuantaCann2™, and also by HPLC. The four plots that follow show a comparison of the NIR predicted value on the Y-axis, plotted against the HPLC-measured data for the same sample on the X-axis. Ideally, a straight line with slope = 1 is obtained. Statistical scatter is, as expected for all measured data, observed and is evident on the plots by deviations between the actual points and the line of slope 1. The absolute percent errors are all about 1%. That means, if the QuantaCann2™ predicted value obtained for THCA is 18%, then HPLC analysis of the same sample would likely yield a value in the range 17-19%. In fact, this approaches the precision of HPLC for repeated measurements of the same plant sample.

How Accurate is NIR for CBDA, CBD, THCA & $\Delta 9$ -THC?

The NIR analyzer can measure CBDA to 0.7% accuracy, CBD to 0.4% accuracy, THCA to 1.3% accuracy and $\Delta 9$ -THC to 0.6% accuracy (RMSE). Specific prediction comparisons versus HPLC reference data are presented in the following Figures 1-4:

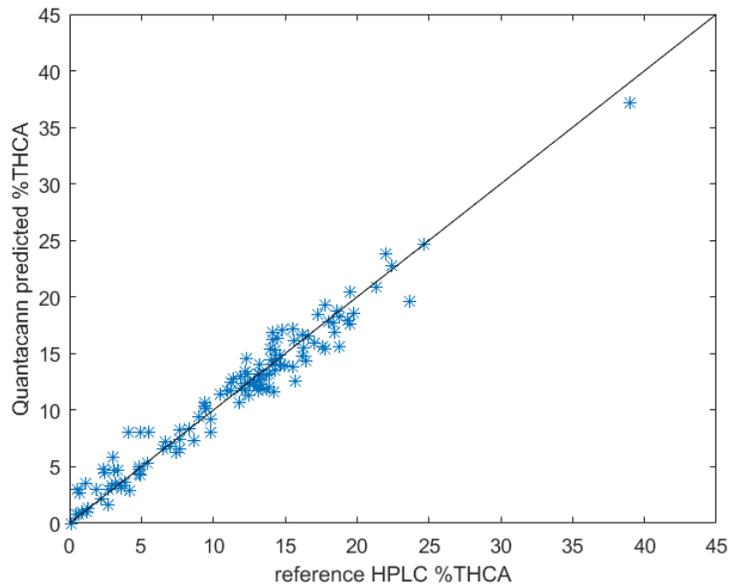


Figure 1: THCA comparison. NIR prediction (Y) vs HPLC measurement (X). The solid line indicates a perfect match. Data shown are for samples not included in the training calibration. The value near 40% is a flower sample enhanced with added THCA using concentrate oil and kief.

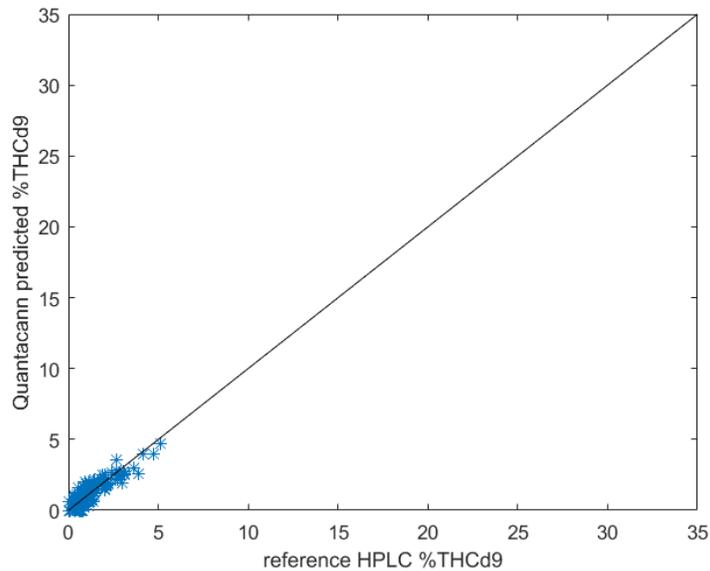


Figure 2: $\Delta 9$ -THC comparison. NIR prediction (Y) vs HPLC measurement (X). The solid line indicates a perfect match. Data shown are for samples not included in the training calibration. Cannabis plants produce mostly acid cannabinoids, so the range of THC neutral in flowers is much smaller (0-5%) than for THCA.

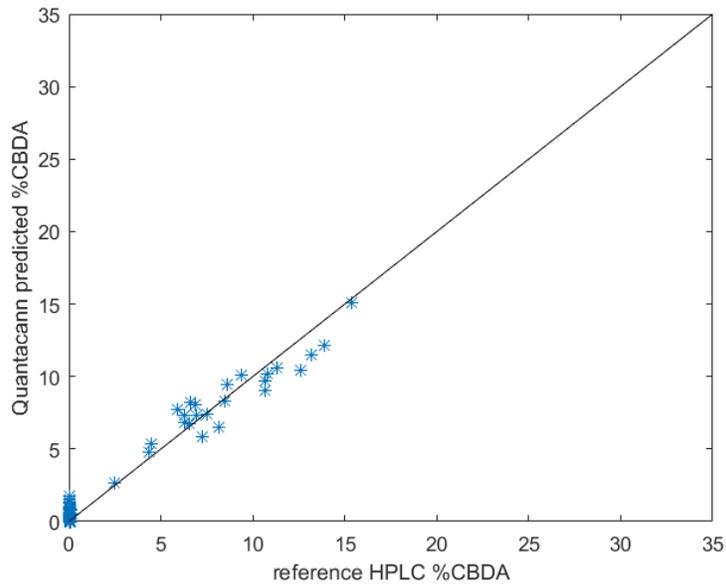


Figure 3: CBDA comparison. NIR prediction (Y) vs HPLC measurement (X). The solid line indicates a perfect match. Data shown are for samples not included in the training calibration. Most flowers that produce significant THCA do not produce much CBDA. Flowers that produce large amounts of CBDA are more rare, so there are fewer samples with high CBDA, but lots with very low CBDA.

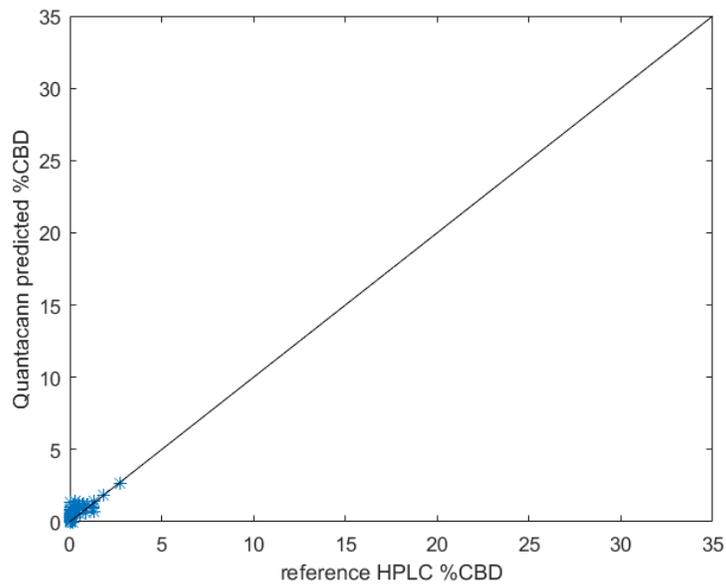


Figure 4: CBD comparison. NIR prediction (Y) vs HPLC measurement (X). The solid line indicates a perfect match. Data shown are for samples not included in the training calibration. Cannabis plants produce mostly acid cannabinoids, so the range of CBD neutral in flowers is much smaller than for CBDA. Many samples have almost none.

Why (Not) Analyze for CBN?

Near IR is not competitive with wet methods in analysis of minor residual components such as pesticides, and low concentrations of cannabinoids. The NIR is not capable of measuring CBN accurately because it exists in very low, insignificant concentrations in cannabis flowers. Wet method data collected at Steep Hill shows that >99% of all cannabis samples have less than 0.25% CBN and the average quantified value is 0.07%. Below is a histogram of CBN contents of 6,500 flowers tested at Steep Hill in California, Washington, and Colorado.

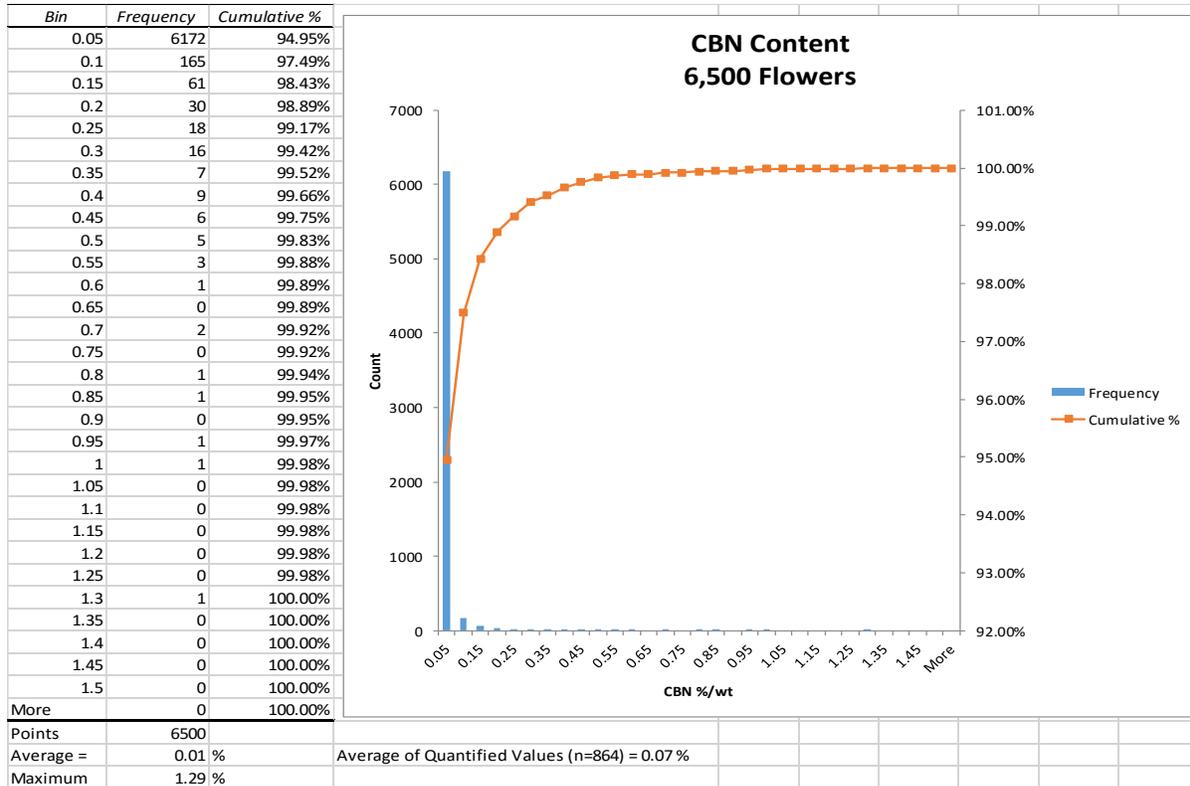


Figure 5. Histogram of CBN concentrations of 6,500 flower samples. Data is from flower sample tests performed by Steep Hill in its California, Washington, and Colorado laboratories using HPLC-UV-PDA methodology. Over 85% of flower samples contained too little to report (only trace amounts), and >99% of all samples tested have less than 0.25% CBN.

So the question arises: Why test for CBN? When Steep Hill first started testing cannabis commercially in 2008, CBN was one of only four cannabinoid standards available and was included with CBD, $\Delta 9$ -THC and $\Delta 8$ -THC (the latter of which is not found in plants) in the test protocols and results. There was some thought that the amount of CBN present could be a measure of how old the flowers were. However, this has not proven to be the case. Amounts of CBN in cured flowers are always exceedingly small and depend on many details of growing, curing, and storage. Regulations requiring CBN determinations in cannabis likely will leave wet methods and the complexity and cost of a wet lab as the only choice for cannabis potency analysis. The cost, efficiency and environmental benefits of a rapid analyzer should be weighed against the desire to determine such low levels of CBN and the associated need for wet analysis of every sample.

Moisture Reduction Helps Prevent Mold Growth

Steep Hill is firmly committed to helping ensure the safety of the legal cannabis supply chain. While the QuantaCann2™ cannot measure extremely low levels of microbiological and chemical compounds (i.e., parts-per-billion levels of pesticides, mycotoxins, etc.), it does aid in reducing mold occurrences in flowers by helping to identify (and thereby control) moisture content. In fact, Steep Hill's QuantaCann2™ measures moisture content more accurately than most laboratory moisture testing processes. The most commonly used lab moisture testing method measures simple weight loss after heating by: weighing sample, then heating sample to evaporate water (which also unintentionally drives off other volatiles), weighing sample again, and then attributing the mass difference to moisture. However, since cannabis flowers contain many volatiles (such as terpenes and flavonoids in addition to water) at low percent levels, measuring mass loss after heating necessarily overestimates the true water content, often by a factor of 2 (volatile terpenes can easily measure 5-10% by weight in freshly cured cannabis flowers). Instead, Steep Hill uses a proprietary method using mass spectrometry detection of water (with no chance of interference from terpenes or other volatiles) to calibrate the QuantaCann2™ for the detection of moisture content. Flowers that contain excessive amounts of moisture (>~8% moisture content by weight) are much more likely to develop mold infestations than are properly dried and cured flowers (<~7.5% moisture content). Accurate moisture measurements provide the opportunity to accurately gauge the efficacy of the curing process for each sample and allows the producer to correct an excessive presence of moisture in their flowers before it leads to mold infestation.

Scientific Personnel

Steep Hill's QuantaCann2™ development has been conducted by two highly experienced PhD Analytical Chemists.

Dr. Donald Land is Professor of Chemistry at the University of California, Davis where he builds and uses spectrometers of many types and is also a founder of Halent Laboratories, a cannabis testing firm that merged with Steep Hill in 2013. Dr. Land has pioneered the development of wet lab cannabis testing methods for over 6 years, first at Halent and more recently at Steep Hill. Dr. Land is also a professor of forensic chemistry with special expertise in infrared spectroscopy and mass spectrometry. Development of a cannabis analyzer requires high quality reference data in modeling and Dr. Land's experience in cannabis lab analytics enables the collection of the highest quality reference data.

Dr. Thomas Blank is an analytical chemist with 20 years of spectroscopic applications development experience including NIR/chemometrics modeling in the oil industry where he was a post-doctoral fellow at Exxon Research and Engineering. Dr. Blank has deployed and tested more than 50 refinery models for gasoline and diesel blending using mid/NIR analyzers while at Exxon. Dr. Blank also has 12 years in medical device testing of NIR and Raman spectroscopic instruments and air quality testing analyzers based on Near and Mid IR spectroscopy.

Links

NIR in the Oil and gas industry:

<http://www.asdi.com/getmedia/111f2a9e-45e5-4bc4-849a-cbb281df6a14/Near-Infrared-Spectroscopy-finding-use-in-energy-industry-Oil-Gas-Product-News1.pdf.aspx>

<https://www.osapublishing.org/as/abstract.cfm?uri=as-55-2-197>

<http://www.ogj.com/articles/print/volume-91/issue-18/in-this-issue/refining/near-infrared-offers-benefits-and-challenges-in-gasoline-analysis.html>

<http://www.ogj.com/articles/print/volume-92/issue-26/in-this-issue/general-interest/experience-leads-to-accurate-design-of-nir-gasoline-analysis-systems.html>

NIR observed degradation of grains:

<http://naldc.nal.usda.gov/download/56644/PDF>

<http://naldc.nal.usda.gov/download/12144/PDF>

<http://pubag.nal.usda.gov/pubag/downloadPDF.xhtml?id=23127&content=PDF>

Use of NIR in Agriculture, book:

<https://dl.sciencesocieties.org/publications/books/articles/agronomymonogra/nearinfraredspe/frontmatter>

NIR in nutrition content of meals:

<http://pubag.nal.usda.gov/pubag/downloadPDF.xhtml?id=17538&content=PDF>

NIR and applications, general overview:

http://www.jsac.or.jp/analsci/data/pdf/28/06/a28_0545.pdf

<http://www.spectroscopynow.com/details/education/sepspec1881education/an-introduction-to-near-infrared-spectroscopy.html?tzcheck=1,1,1,1,1&tzcheck=1>

Analysis, Publishing & Copyright

This analysis has been jointly conducted by Dr. Donald Land and Dr. Thomas Blank and the report is being published on June 7, 2016.

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