The Use of Near Infrared Spectroscopy for Potency Measurement

Introduction to the Luminary™ Profiler

Outline

I. Background
   a. State of cannabis industry
   b. Why is potency measurement needed?
   c. State of the art for potency measurement

II. Why is optical spectroscopy the preferred solution for cannabis potency testing?
   a. What is Near Infrared Light (NIR)?
   b. Introduction to the Luminary Profiler
   c. Why is NIR preferred?
   d. FDA Guidance Supporting Optical Technology for Process Analytics and Testing (PAT)
   e. Benefits of Using NIR in Industry

III. Sampling

IV. Application
   a. Using the Luminary™ Profiler
   b. Model Development for Accurate Predictions of Cannabinoid Potency

I. Background

a. State of Cannabis Industry

The evolution of the cannabis industry in the U.S. has been far from ordinary. Marijuana currently remains a Schedule I drug under the federal Controlled Substances Act of 1970, and thus illegal for any reason under federal laws, with the exception of FDA-approved research programs. Notwithstanding, states have taken steps to legalize the substance at a slow and steady rate since 1998, when voters passed approval of medicinal marijuana, and in the past five years, it has accelerated to 35 states having some form of legalization. As of November 2014, four states and the District of Columbia have legalized "adult use" or recreational marijuana, all through voter initiatives as opposed to legislative mandate.

Because of this slow and awkward growth of the quasi-legal market for marijuana, complementary markets have been understandably reluctant to organize. For this reason, development of the testing technologies focused on the applications of potency and safety measurements of cannabis have been relatively stagnant.

b. Why is Potency Measurement Needed?

Potency, in the traditional pharmaceutical sense, means the amount of active ingredient in a product. In cannabis, potency is driven by tetrahydrocannabinolic acid, THC, which is its primary and most prevalent psychoactive compound. In flowering products, THC content is generally around 10 to 20% by weight. In hash and extracted oils, this content can be much higher (up to 80% or more by weight). The second element of cannabis potency is cannabidiol, CBD, which does not provide the psychoactive stimulation of THC but, rather, physiological relaxation associated with therapeutic usage of cannabis (anxiety relief, muscle relaxation, appetite stimulation, among others). CBD is typically present in much smaller concentrations, less than 10% by weight and usually closer to 1 or 2%. The third component of potency is cannabinol, CBN, which is a very weak psychoactive compound and, importantly, a natural breakdown product of THC that occurs over time. In fresh product, CBN is typically at or near 0, but this percentage increases as the product slowly degrades over time. As such, CBN can be a useful indicator of the product freshness. While multiple other cannabinoids are present in cannabis, these three are the most widely studied and ascribed physiologic importance. Future scientific studies may reveal significant effects from the remaining cannabinoids, in which case their inclusion in the overall measurement of "potency" will be considered.

There are thousands of unique strains of cannabis being grown and consumed, yet the ability to present an accurate potency measurement for each has been severely limited due to the costly, and relatively inaccessible methods that have been available for strain testing. Consequently, marijuana

users have not been assured of receiving an accurately labeled product, or of product consistency over multiple purchases. While frustrating for recreational users, it can mean devastation for medical marijuana patients whose abatement of painful symptoms depends on the critical need for both accuracy and consistency.

c. State of the Art in Cannabis Measurement

The state of the art in cannabis chemical characterization entails established laboratory analytical techniques, including gas and liquid chromatography (GC and LC) and mass spectrometry methods. While these methods provide accurate molecular characterization and quantification, there are many drawbacks that impede its comprehensive adoption, and prevent it from enhancing the productivity of the rather unique nature of the cannabis industry. For example, GC and LC methods typically require sample preparation and destruction, the use of consumable preparatory materials, a skilled operator, and/or a waiting period of one to more typically several or more minutes to obtain a result. This timeframe is incorporated into the overall several days that it takes a grower, dispensary or regulator to ship the samples and await the results from the remote testing facilities that provide potency data. Further, hazardous waste is often a by-product of GC and LC testing methods. To date, it has been necessary for cannabis samples to be sent to dedicated laboratories, a relatively expensive and time consuming process, thus creating a bottleneck to optimal productivity of cannabis industry operations.

Because of these impediments, regulators have only required a small sample or a larger lot to be actually tested. However, due to the natural variations inherent in such an organic plant substance as cannabis, the true potency of each product of the lot that gets consumed but was not actually tested cannot be known for certain by the user.

It is important for customers to have accurate potency measurements of the medical and recreational cannabis products they are purchasing. Additionally, the still nascent regulatory climate is maturing rapidly and will predictably reach the levels of stringency required for alcohol and pharmaceuticals. Testing procedures that are more flexible, practical and affordable will facilitate these needs and developments.

II. Why is Optical Spectroscopy the Preferred Solution for Cannabis Potency Testing?

Optical techniques such as reflectance and absorption possess several hallmark advantages over conventional analytical techniques for chemical characterization and quantification. Optical methods are nonintrusive to the sample, which allows measurement without altering the chemical content or causing any physical change to the sample. Other benefits of optical spectroscopy include: allowing rapid sample measurements (typically in less than one second), molecular specificity, such that individual compounds of interest can be identified distinctively, typically without any further sample preparation, automated characterization or quantification, allowing their use by unskilled personnel or with minimum training, portability for use on-site and with as much frequency as desired, and lastly, use of devices that require little or no maintenance over their operating lifetime.

a. What is Near Infrared (NIR) light?

Visible light, which ranges from about 400 to 800 nm, contains almost no chemical information and is actually a measure of a material's color. Near-infrared (NIR) light is the region of the electromagnetic spectrum just beyond the red light that our eyes can see, around 800 nanometers (nm), and extends to approximately 2,500 nm. This wavelength range has become increasingly popular for rapid chemical assessment in a number of industries, because it contains a wealth of information and can be measured very quickly. Light in this region of the spectrum interacts with the chemical bonds in molecules. By measuring the light intensity returned from the samples at each point in the spectrum, characteristic fingerprints can be accurately and rapidly measured. NIR can be used to monitor both chemical and physical properties, and has been frequently employed for qualitative analyses in the food, chemical, oil, gas, petrochemical, feed, agriculture and pharmaceutical industries.

b. Introduction to the Luminary™ Profiler

The Luminary™ Profiler developed by Sage Analytics (sageanalytics.com) uses the most information-rich portion of the NIR range (~1500 to 2000 nm), where chemical features are significantly more pronounced and immune to unimportant factors like the cannabis color. This enables a more precise measurement of the cannabinoids and product moisture. Diving deeper into the science, the NIR spectral region from approximately 700 to 2500 nm corresponds to overtones and combination bands of molecular vibrational absorption. The functional groups probed by NIR are predominantly those containing hydrogen bonds. Compared to mid-infrared spectroscopy, where fundamental absorptions are evaluated, NIR can be used to measure thick samples with high water content, and its allowance for diffuse scattering permits its use in both transmission and reflectance geometries, vastly simplifying sample preparation and measurement.

While NIR light is becoming more prevalent in various industries, the key enabler to its use is the ability to translate the NIR information into a meaningful value. In this case, the meaningful values are the cannabinoid quantities present in tested samples. Just as they have done for determining the exact amount of active drugs in pharmaceutical tablets as they are being produced, Sage Analytics' scientists first characterized the NIR signature of the purified cannabinoids. This was done through a unique agreement with leading academic researchers, as such substances require precise scientific procedure, knowledge and institutional support to develop. From these cannabinoid signatures, sophisticated mathematical models were developed to accurately quantify the amount of each specific cannabinoid in the total NIR signal recorded from a tested product, regardless of product strain or type (or color). This is the same approach approved by the FDA for NIR use in pharmaceutical testing, and is a robust method to test any form of product.

Cannabis is a natural product, however, and like all natural products the variation is enormous: regions of the same bud will have different potency, as will different buds from the same plant, buds harvested at different times, buds stored for different amounts of time, etc. Since the Luminary™ Profiler only measures the NIR light signature of the cannabinoids however, none of this variation affects its measurement. The Luminary™ Cloud is used to access the set of mathematical formulas that translate the new sample's light signature into accurate cannabinoid potency metrics. These algorithms are stored in the cloud so they can be continually improved, and to add further test parameters (*e.g.* additional cannabinoids and chemicals) as the Sage scientists hone their testing accuracy in their controlled laboratories with rigorous procedures, not using the samples sent in by users.

It is important to note that NIR spectroscopy, as employed in the Luminary™ Profiler, is considered a secondary analytical technique, whereas GC and LC are primary methods for evaluating cannabinoid potency. This simply means that the Luminary™ Profiler is reliant on a cannabinoid potency data set that has been previously characterized using a highly accurate, primary method for the development of models capable of calculating potency.

c. Why is NIR, and the Luminary™ Profiler, preferred for potency measurement?

Each participant of the cannabis market eco-system (growers, dispensaries, regulators, marijuana-infused product manufacturers) has an important need to ensure accuracy and consistency of their cannabis potency measurements, this necessity has been hindered by the inherent obstacles of, and omissions to current testing methods. Specifically, until now, nearly all testing must be conducted off-site of a business or regulators' operations. This burden causes delay, and because of this delay, one's operations are prone to error or inconsistency. Safety is an issue, as the need to transport marijuana samples having such a high monetary and black market value can present a risk to the transport agent. The sampling of cannabis plants in the field can lead to contamination of the samples to be evaluated, which can result in errors in the analysis. This contamination may stem from, for example, extraneous

debris adhering to the harvested plant material. Most notably, if on-site testing was available on a cost-effective basis, this would enable quality control and production efficiencies to increase considerably. Additionally, costs, delay and transport risks could be significantly reduced.

The Luminary™ Profiler enables potency measurements in a diverse assortment of cannabis-based products including flowers, kief, hash, oil, waxes, concentrates, etc. The majority of these products require no sample preparation, significantly decreasing experimental time and cost. The samples can be evaluated more rapidly, allowing a greater quantity of samples to be measured. This feature of the Luminary™ Profiler translates to the proficiency to analyze a greater proportion of available samples, as opposed to current methodologies, where a sample subset is deemed representative of the whole. This means that vast quantities of potential products are not independently analyzed due to the cost of such an endeavor. By analyzing more samples in less time, the Luminary Profiler provides the potential to exhaustively assess cannabinoid potency in *all* samples, leading to more detailed knowledge of the chemical composition of a cannabis strain, and therefore, more accurate labeling. Further, use of the Luminary Profiler enables existing GC/LC equipment at laboratories to be freed up to increase throughput of contaminant testing.

d. FDA Guidance Supporting Optical Technology for Process Analytics and Testing "PAT"

In 2004 guidance was provided by the Food and Drug Administration (FDA) to the pharmaceutical industry, titled "Guidance of Industry, PAT (Process Analytical Technology) – A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance". This initiative has helped process improvements evolve in an otherwise risk-averse and reluctant industry and governing regulatory body. As changes to the testing and regulatory processes in the pharmaceutical industry were stymied from fear of process deviations, the FDA recognized it was missing opportunities for technological improvements, and thus implemented instructions to facilitate innovation and technological advances to enable production process improvements. The FDA guidance was warmly received, and was successful in increasing innovation of PAT including the use of NIR for analytical processes. NIR is the PAT instrument of choice encouraged by the FDA. Since the PAT initiative was unveiled over ten years ago, the advances that have been enabled are credited with causing a substantial decrease in the time required to bring drugs to market, significantly improving quality control in manufacturing, and cost savings throughout the pharmaceutical industry.

As the cannabis industry evolves through the legalization process, the coordination of its regulation follows. Indeed, the need for standardization throughout the marijuana industry is dire, as states are dealing with regulatory issues independently and in serial fashion, without a federal agency to oversee the efforts. Needs addressed in the FDA PAT Guidance include advances in process analyzers to make real-time control and quality assurance during manufacturing feasible (as opposed to off-line and remote testing); avoiding the need for sample preparation; and for integrated systems. Goals include

improving the scientific basis for regulatory specifications; promoting continuous improvement to ensure precision and consistency of production; and improving manufacturing while maintaining or improving current levels of production growth. This guidance is highly relevant to the similar needs and goals of the cannabis industry, and thus should be considered as a precursor to the regulatory guidance and rules that will inevitably continue to refine and evolve to ensure the safety of the public.

e. Industry benefits of NIR

There are many benefits that NIR spectroscopy, as used in the Luminary™ Profiler, can provide over traditional GC and LC techniques, and that are specific to the unique requirements of the evolving cannabis industry. NIR benefits include:

- Simplifies obtaining measurements of potency and moisture through highly accurate THC, CBD and CBN authentication at each point in the cannabis growing, production, regulation, distribution and sales ecosystem, without requiring a trained technician to process the testing and results.
- Provides a portable, on-site device for use by nearly every business in the cannabis supply chain.
- Provides in-process and instantaneous, highly accurate potency and freshness profiles for flowers, and potencies of concentrates and infused products, that can be viewed on a display screen or printed on a label or receipt.
- Are easily operated by any employee or customer; does not require a skilled operator.
- Rapid acquisition of data as opposed to standard LC and GC methods.
- Does not chemically alter the sample to be analyzed.
- Tests the samples without causing their destruction.
- Does not produce any hazardous waste byproduct.
- Retains the measurement results for a company's sole access for use in quality control and archive purposes.
- Reduces the cost and complexities associated with remote lab testing by ensuring operations, and samples therefrom, are in optimal conformance with regulatory testing.
- On-line attributes reduce the risk of contamination through harvesting and transporting samples from the field to the lab.
- Increases safety of operations by requiring less transport of samples.
- Better flower or plant averaging by acquiring data from multiple plants and portions thereof, resulting in more realistic potency measurements.
- Greater accessibility to consumers by providing the ability to test and measure each product

purchase, thus increasing consumer safety through greater accuracy for both Adult Use customers and Medical Marijuana patients.

As applied to the cannabis industry ecosystem, the benefits attained from the Luminary™ Profiler products extend across most participants:

- Regulators and Government Officials can test instantly, on-site, and on their own, for active ingredient levels of THC, CBD, and CBN without relying on delays associated with remote lab testing – inexpensive, accurate testing is now available at their fingertips.
- Growers may regularly monitor potency of their crops to determine the optimal time to harvest, resulting in enhanced quality and consistency of the product. Potency levels of the flower may be verified while it remains on the plant.
- Laboratories are able to increase testing yields, significantly reducing the testing turn around time, and lowering the skill requirements for operators to measure THC, CBD, and/or CBN levels.
    - Existing labs will increase their businesses through these higher-throughput tools over existing, more complex GC and LC equipment (which can be used for other types of testing such as for contaminants).
    - Lower cost of entry and skill requirements enable more labs to be formed.
- Manufacturers of edibles and infused products can test potencies of concentrates and butters before processing them to assure desired and consistent cannabinoid levels in every batch.
- Medical marijuana dispensaries can offer a faster and less costly, yet accurate and reliable method to provide optimal THC and CBD ratios for their patients, at their facilities, and at the point of sale.
- Dispensaries have real-time point-of-sale measurements that enable their retail customers to have the assurance that the potency and freshness levels in flowers and extracts are accurate -- providing them confidence in the purchase of
the product.
    - Product labels or receipts can be printed instantly upon testing while observed by the customer / recipient.
- Customers obtain more consistent and precise potency products.

III. Sampling

A major limitation of traditional analytical tools for evaluating cannabis potency is that the plant samples must be subjected to an extraction to remove the cannabinoids from the complex chemical matrix that composes the whole plant. These extractions often employ solvents, such as chloroform and methanol, which are health hazards, and require distinct safety precautions to be implemented. Additionally, if GC is used to measure potencies, the molecules must be volatile (meaning readily converted from a liquid to gaseous state). This means that the acidic forms of THC and CBD cannot be measured with GC, unless they are derivatized with a molecule that makes them more volatile. This added sample preparation requirement decreases the number of samples a researcher can measure in time. As previously mentioned, GC destroys the sample, and retrieval of the compounds of interest in LC would require costly and laborious purification.

The facility of the sample preparation using the Luminary™ Profiler enables researchers to spend more time evaluating samples, rather than time-consuming, potentially toxic preparatory steps. The Luminary Profiler can measure samples in any form (i.e., solids, oils, waxes). Whole buds can be used, however Sage Analytics' scientists recommend that the samples be homogenized through grinding, to provide a more accurate and realistic measurement of cannabinoid levels throughout the bud. For example, trichomes contain high THC levels, and if the user honed in on this area of the plant, falsely high THC contents may be reported. Likewise, if the sample contains stem material, and the NIR light probes this region of the sample, anticipated cannabinoid levels will be skewed.

IV. Applications

a. Using the Luminary™ Profiler

The ease of use is one of the foremost features of the Luminary™ Profiler. Each system includes a detailed step-by-step software wizard to ensure that users feel comfortable in operating the instrument. A list of the main steps in acquiring cannabinoid potencies using this system is provided below:

- Calibration
    - When initially turning the system on, the Luminary Profiler requires a 20-minute warm up period to ensure that the lamp reaches a stable operating temperature. The system need only be turned off when it will not be used in order to save lifetime of the bulb.
    - The measurement window must be thoroughly cleaned with isopropyl (rubbing) alcohol wipes. The alcohol should be allowed sufficient time to completely evaporate, as it in moist form can potentially interfere with the potency analysis.
    - After cleaning the window, the system must be calibrated using two references, entitled "black" and "white" calibrations. These calibrations steps are mandatory when the Luminary Profiler is first powered on, and every 24 hours thereafter (once daily). They do not need to be performed before measuring every sample.
    - The black calibration cap is placed over the cleaned window. This provides a measurement of any signal produced by the instrument in the absence of NIR light and any samples.
    - The white calibration uses a National Institute of Standards and Technology Spectralon® reference puck. This is a completely reflective surface, and as the black calibration provided a lower limit of the spectral signal, the white reference provides the upper limit of a maximum amount of light coming into the measurement window. The white reference puck is encapsulated in glass, and therefore, should not be touched or scratched. The puck should be thoroughly cleaned, as needed, with the rubbing alcohol wipes.
- Measurement of Cannabinoid Potency in Dry Samples
    - The Luminary Profiler can be used to evaluate whole or ground dry cannabis samples. It is recommended, however, that the samples be ground to homogenize the plant matter and achieve more reproducible measurements.
    - After the sample window has been cleaned with the rubbing alcohol wipes, the dry plant material should be placed on the measurement window in such a way that the entire

window is covered. This will ensure that there are no regions where no sample is being evaluated by the NIR light source.

- After filling the measurement window with the sample, the black calibration cap is placed over the window to ensure that no stray light enters the system.
- The user then presses the Calculate button, and after approximately 10 seconds, the potency values for that specific aliquot of sample are provided.
- The user should remove the entire sample from the measurement window, and thoroughly clean the window with the rubbing alcohol wipes prior to measuring additional samples. Once the alcohol has evaporated, the next sample can be applied to the window.

- Measurement of Liquid Samples
  - The Luminary™ Profiler comes equipped with an extract ring for the measurement of liquid samples, such as oils and concentrates. After following the calibration protocol, the user can select this type of sample.
  - The extract ring must be thoroughly cleaned using the rubbing alcohol wipes. Prior to adding the sample, the ring must be measured to ensure that no NIR signal is produced from the ring and the external reflector. Place the ring on the measurement window, and then cap with the sample/black calibration cap, with the *silver* side facing down. Calibrate the instrument using this sample holder configuration.
  - Add the liquid sample to the extract ring using a wide-orifice syringe. The user should add enough liquid to fill the sample well, and the sample should completely cover the measurement window. Failure to completely cover the measurement window may lead to analysis error. This procedure is predominantly for translucent samples. If a sample is dark or opaque, it may be necessary to add a thinner disc of sample to the measurement window, as highly darkened samples can prevent the reflected light from passing through the measurement window. In these instances, the measurement window should still be completely covered.
  - After adding the sample to the measurement window, the external reflector (silver side of the black cap) is placed over the window. Since many of these types of samples are translucent, the NIR light will pass through the sample. In order to have the transmitted light reflect back through the measurement window, a reflective surface is needed. This process is termed “transflectance” since the light originally was transmitted through the sample, and then reflected back through the measurement window to be analyzed.

- As many of the liquid cannabis products are quite viscous, it is imperative to exhaustively clean any residual product from the measurement window and the extract ring between sample evaluations.

- Storage and Care of the Luminary™ Profiler
  - The Luminary™ Profiler should be stored in an area free of bright lights and strong airflow, and placed on a flat and stable work surface.
  - The daily calibration of the instrument will aid in ensuring maximum accuracy, and will normalize the system. The calibration should be performed when the instrument is turned on, and at least every 24 hours of continual use.
  - It is acceptable to leave the Luminary™ Profiler on when not in use; however, the Luminary™ Profiler is equipped with a lamp that has a finite lifetime. Therefore, when left on, and not in use, the lamp's operational lifetime will be decreased. Replacement lamps are available from Sage Analytics.

b. Model Development for Accurate Predictions of Cannabinoid Potency

The Luminary™ Profiler combines traditional cannabinoid potency measurements from LC with NIR spectral data to develop multivariate analysis (MVA) models capable of accurately predicting THCa, THC, CBDa, CBD, and CBN contents. When only one analyte is measured, a standard univariate calibration curve is acceptable. Even if there are more than one species being quantified, a univariate calibration may work, if there are unique spectral features to each molecule. This is confounded when there are several analytes with similar chemical compositions, like cannabinoids. MVA enables to simultaneous quantification of all analytes, while significantly reducing the dimensionality of the data. This simply means that MVA reduces the amount of variables (each spectral point, for example) to hone in on those that are paramount to the model's quantitative accuracy. Thus, several thousand wave numbers may be truncated to a few hundred or less, depending on the amount of information the data contains. The data generated from the LC analysis of the samples is united with the NIR spectra from the same strain aliquots.

The spectral data must be preprocessed to remove contributions from physical differences in the plants, such as particle size variation, color, etc., such that only chemical disparities influence the model's development. Once the spectra have been effectively transformed, the model is produced using the reference data (LC) and the NIR transformed spectra. Ideally, the model should contain a diverse assortment of samples that encompass the expected analyte range of future samples. This is a key consideration, as the analyst must prognosticate what type of samples may be evaluated over time. To appropriately construct a model, the reference

samples should be split into randomly generated calibration and validation data sets. Initially, model accuracy can be gauged using a random or full cross-validation, depending on the total number of samples used for calibration. When a particular preprocessing method leads to suitable models (based on a statistical analysis of model performance), the model should be used to predict the cannabinoid content of the validation set. Since the potency values are known for this data set, the analyst can more accurately evaluate the robustness and validity of the model by assessing various metrics including the root mean standard error of cross-validation (RMSECV), the root mean standard error of prediction (RMSEP), the coefficient of correlation (R^2), the slope of the calibration line, the number of factors used to build the model (Scree plot), etc. The RMSEP and the number of factors are two of the most important parameters to monitor when building models. The RMSEP provides the error in the predicted values and also indicates the correct number of decimal places to include when reporting the potency value, as uncertainties should only be reported to one significant figure, since any further digits would be even less certain. If a model has a RMSEP value of 0.05%, the potency should be reported to two decimal places, 0.5%, one decimal place, and 5%, zero decimal places. Choosing an appropriate number of factors allows the most relevant spectral information to be included in the model, without over-fitting the data. When a model is over-fit, it attempts to explain random noise in the spectral data, a deleterious endeavor, as the accuracy of the model will plummet.

Another important metric to consider is the standard error of the laboratory, which is a gauge of the error in the reference method. This value is the lower limit of potential error in the model, as a model cannot out-perform the data used to generate it. Therefore, it is paramount to both know this value, and to recognize that a predictive model will *never* be more accurate than the reference method. The benefit of using NIR spectroscopy to develop models is that many more samples can be evaluated in time, compared to the reference method. This becomes an important point when considering that fact that very little of the bulk cannabis material is tested. In Colorado, for example, one gram out of every pound (454 grams) of cannabis is tested amounting to 0.2% of the product being deemed representative of the whole. The standard methods for analyzing this 0.2% are costly and time-consuming, limiting their utility for evaluating larger quantities of samples. Models constructed from reference and NIR spectral data enable the rapid, cost-effective, and accurate analysis of cannabinoid contents for *all* samples, providing a more realistic evaluation of the chemical constituents in cannabis strains as well as providing patients and recreational consumers a greater awareness as to what they are ingesting and inhaling.