The Quest for "True North" in Measuring Cannabis Potency

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As the medical cannabis industry has evolved, it has become apparent that there are various analytical methods to evaluate the cannabinoid content, or potency, of different strains. Knowing the cannabinoid content levels present in flowers, extracts/concentrates, and marijuana- infused products (MIPs) allows consumers to make educated decisions when purchasing products. However, laboratories use differing techniques such as high-performance liquid chromatography (HPLC) or gas chromatography (GC), leading to variable measurements of tetrahydrocannabinol (THC), cannabinol (CBN), and cannabidiol (CBD). In addition to the adoption of different instruments, analysts have varied experimental protocols within a defined analytical method. For example, in LC, technicians must select an appropriate column, eluent, flow rate, experimental run time, internal or external standard(s) to evaluate instrumental performance, and a suitable concentration range for measuring calibration standards, to name a few. The same experimental parameters must be selected in GC, as well as an appropriate carrier gas. Thus, the experimental protocols have become diverse, with different labs adopting different cannabinoid analysis strategies. This lack of standardization has led to the inability to actively compare cannabinoid metrics measured from different labs, necessitating a standardized protocol to be nominated and implemented henceforth. The adoption of a standardized method will allow scientists to contrast results between labs, and will enable round-robin style evaluations of the same sample to gauge the validity of the method, and potential lab-to-lab operator error. This method will also enable cannabis patients to select products in the same way they might choose an over-the-counter medication or other pharmaceutical products.

LC has been routinely used in industrial settings to separate and quantify analytes in a complex mixture. Samples must be in liquid form, necessitating the efficient extraction of compounds of interest from solid samples. A benefit of LC over GC is that the natural, acidic forms of THC and CBD can be quantified without requiring the derivitization of these molecules (as in GC) to enhance volatility. A downside of adopting LC over GC is the broader peak widths, resulting in decreased resolution of peaks with similar retention times. As mentioned above, there are various experimental parameters that must be optimized to obtain high peak resolution, such that samples with similar retention times can be differentiated and accurately quantified. These experimental conditions lead to some inherent shortcomings when seeking to measure a large volume of samples. For example, in the American Herbal Pharmacopeia, the run time chosen to provide the best separation of individual cannabinoid peaks was 30 minutes plus a six minute post-run to flush the column. If samples were measured using three representative aliquots per sample of interest, one sample would take 108 minutes. In addition, in order to obtain the isolated cannabinoid samples, the flower (bud) must be efficiently extracted. The extraction solvents employed (i.e. chloroform, methanol) are health hazards, requiring proper safety

precautions to be adopted. Additionally, the eluent (acetonitrile and ammonium formate, for example, in the American Herbal Pharmacopeia) must be properly disposed. Thus, the laborious sample preparation, long analysis times, and use of consumables limit the cost- effectiveness of using LC for analyzing thousands of samples.

GC techniques possess many of the same limitations when evaluating multitudes of samples. Additionally, unlike LC, GC cannot quantify the acid forms of THC and CBD, since the temperatures used decarboxylate the acid, forming neutral THC and CBD. Thus, the THC measurement provided is a gauge of the total THC contained in the plant (acid and neutral forms). GC is a destructive analytical technique, as the sample is converted to a gas prior to analysis. This feature translates to the loss of a valuable commodity, in order to obtain cannabinoid potency. The destruction of an aliquot of the sample could result in cannabis product vendors limiting how many samples they measure, leading to an erroneous representation of whole batches of sample by analyzing a small subset. The use of compressed gases presents a safety concern when using GC methods, and users must be extensively trained on how to safely use the gas cylinders, including how to change the regulators, how to properly store the cylinders, etc.

An underlying common theme when assessing the standard methods is that, although they are invaluable for analyzing smaller sample sets, their limitations prevent their use when higher throughput methods are desired to exhaustively evaluate cannabis samples. Vibrational spectroscopy has been routinely shown to relinquish the limitations of the standard methods when measuring plants (Lupoi et al., *Bioenergy Research*, 2014; Lupoi et al., *Biotechnology for Biofuels*, 2014; Lupoi et al., *Bioenergy Research*, 2015; Templeton et al., *Cellulose*, 2009; Shenk et al., *Practical Spectroscopy*, 2008; Ye et al., *Bioresource Technology*, 2008; Yamada et al., *Holzforschung*, 2006). Vibrational spectroscopy studies how light interacts with the sample of interest, and includes mid-infrared (MIR), near-infrared (NIR), and Raman spectroscopy. These methods are non-destructive, enabling users to retain their samples following the analysis. Another attribute of these techniques is the limited-to-no sample preparation requirement for obtaining the data. The high-throughput capabilities of these instrumental configurations enable researchers to thoroughly measure larger sample sets in less time and at decreased costs.

In order to take full advantage of the high-throughput characteristics, researchers often use spectroscopy in conjunction with one of the standard techniques to develop multivariate analysis models that are capable of predicting the analyte of interest accurately and robustly. For example, the Sage Analytics Luminary Profiler coupled GC data with NIR spectra to produce a partial least squares regression model for cannabis potency quantitation. These models are analogous to standard calibration curves, except they contain all of the important variables. Multivariate analysis enables the efficient mining of the spectral data to extract the useful

information that may be obscured when visually analyzing the spectra. The following scenario can exemplify this process. If a lab has 2500 samples to evaluate, whether cannabis, aspirin, cereal grains, etc., the analysis via solely the standard methods would be a laborious and costly endeavor. The sample set can be divided such that the standard methods will be used to evaluate possibly 500-1000 of the samples. These samples are called calibration samples. NIR spectral data is then obtained for all 2500 samples, and the spectral data collected for the 500-1000 calibration samples is coupled to the standard results (i.e., GC or LC cannabis THC percent). After efficiently validating the models using a variety of statistical metrics, and ensuring the model is accurate, the remaining 1500 samples can be predicted. The root mean standard error of prediction (RMSEP) is the uncertainty to be applied in conjunction with the predicted values, and can be compared to the standard error of the laboratory (SEL), which is a metric indicative of the error in the standard method. Ideally, these two metrics should be close in magnitude, which translates to an accurate model that can be used confidently to quantify future unknown samples by merely acquiring the NIR spectrum, and inserting it into the model.

The cannabis industry should be subjected to the same rigorous testing procedures consumers have come to expect for any commodity. Would a consumer be comfortable purchasing a bulk supply of medicine, in which only one per every 1000 samples were analyzed? Even if 10, or 100 samples were deemed “representative of the whole”, can companies providing products expect consumers to blindly trust that a part really represents a whole? The Green Standard Working Group seeks to alleviate this conundrum by nominating a standardized testing method, such that large quantities of samples can be evaluated, giving a more realistic picture of cannabinoid potency across different strains. A standardized protocol would also enable labs to directly compare results to gauge natural sample variation, operator error, etc. Ideally, this method would incorporate an analytical tool, such as the Luminary Profiler, that can enable the whole cannabis eco-system (grow-houses, product manufacturers, dispensaries, and labs) to evaluate *all* samples that pass into their domain. The nomination of a standardized method will lead to a more proficient labeling of products, giving consumers detailed knowledge of the medicines and products they are purchasing, as they have come to expect and demand in as diverse of a portfolio of products such as peanut butter, Sambuca, or aspirin.