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Non-destructive measurement of Tetrahydrocannabinol (THC) and Cannabidiol (CBD) using near-infrared spectroscopy

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Abstract Tetrahydrocannabinol (THC) and cannabidiol (CBD) are cannabinoids which produced by cannabis plants and major compounds found in cannabis products. A predictive method for non-destructive quantification of THC and CBD using near infrared spectroscopy (NIR) technology is developed. The prediction model for THC estimation had coefficient of determination (R-squared) and root mean square error of calibration (RMSEC) values of 0.9994 and 0.1926, respectively. The correlation between THC values of HPLC measurement and NIR prediction showed a correlation coefficient of 0.9078. For CBD prediction, the R-squared and RMSEC values of CBD equation were 0.9995 and 0.0006, respectively. The predicted and measured concentrations of CBD showed good correlation with a regression correlation of 0.9413. The test indicated NIR could be a promising alternative method for THC and CBD evaluation.

Keywords: NIR, Cannabinoid, THC, CBD

Introduction

Cannabis sativa L. is an annual herbaceous species. Cannabis plants have been used for food, fiber, and recreational purposes and recently, it has become widely applied as a medicinal plant. The quality of several herbal products including cannabis products depends upon various factors including raw materials condition that is associated with cultivated varieties, areas, seasons, cultivation, harvest, and postharvest. Cannabis harbors various categories of secondary metabolites including cannabinoids which are the most important substances with recreational and pharmaceutical properties. The major cannabinoids are delta-9 tetrahydrocannabinol (THC), cannabidiol (CBD),

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cannabigerol (CBG), cannabichromine (CBC), and their corresponding acidic compounds such as THCA, CBDA and CBGA. In addition, previous research revealed cannabis provides a wide range of health benefits such as anti-inflammatory, anticonvulsant, and antioxidant (Couch *et al*., 2023; Odieka *et al*., 2022; Boleti *et al*., 2020).

Cannabis cultivars grown in Thailand are well known as 'Thai Stick' that contain high amounts of THC (18-22%). This cultivar is widely used for breeding with other cultivars to produce hybrids. Regarding the enormous number of cultivars, currently, Cannabis is classified by using the ratio of total CBD and total THC. The major three groups are chemotype I, the class with the low value of CBD to THC; chemotype II, CBD to THC ratio is between 0.5 and 3.0; and chemotype III, where the primary content is CBD whilst THC is lower than 0.3%. In addition to these three major groups, the other two groups are chemotype IV with a high amount of CBG, and chemotype V with no cannabinoids. The quality of the medical products of Cannabis is based on the contents of cannabinoids. Because of its psychoactive properties, an amount of THC is legally regulated with various limit values in different countries. Mostly, cannabis with THC above 0.3% is determined as a drug-type. The analysis of THC and CBD content in products is carried out to achieve the standard regulation (Avico et al., 1985).

Nowadays, analytical of medical components in natural products including cannabinoids is conducted by using chromatographic techniques, gas chromatography (GC) with a flame ionization detector, or high-performance liquid chromatography (HPLC) with a diode array detector (Birenboim *et al.,* 2022; Gloerfelt-Tarp *et al.,* 2023). However, the disadvantages of the techniques are time-consuming, laborious, costly, complex operation, and sample destruction. Moreover, the methods involve hazardous solvents such as methanol and acetonitrile. The nondestructive Near-Infrared (NIR) Spectroscopy has been developed and applied to determine the quality and quantity of ingredients of agricultural products. For example, NIR was used to predict maturation of avocado (Olarewaju *et al*., 2016; Melado-Herreros *et al*., 2021), quality of peach (Slaughter, 1995), firmness and sugar content of sweet cherries (Lu, 2001), apples (McGlone *et al.,* 2002; Fan *et al.,* 2009) and satsuma mandarin (Gomez *et al*., 2005), brown heart of pear (Fu *et al*., 2007), and coffee roasting degree (Alessandrini *et al*., 2008). For the quantity aspect, NIR techniques were used to determine concentration of chemical substances in food or natural products e.g., carotenoids in maize (Berardo *et al*., 2004), total anthocyanins, acidity, total soluble solid in grapes (Cozzolino *et al*., 2006), dry matter in 'Hass' avocado (Clark *et al.,* 2003), and gamma-aminobutyric acid content of germinated brown rice (Kaesorn and Sirisomboon, 2014). Cozzolino (2009) reviewed the number of NIR applications to analyze chemical compounds in natural products such as

phenolic compounds, essential oils, and lignin glycosides in green tea and pepper, and sesame seeds. NIR has become an alternative technique with the potential to estimate the quality and quantity of natural raw materials or products. The non-destructive NIR method provides analytical results with accuracy, fast, inexpensive, and environmentally friendly.

In cannabis, Birenboim *et al*. (2022) applied NIR to discriminate cultivar and determine cannabinoids and terpene of inflorescence samples. The other research showed information to support the use of NIR technology for determination of cannabinoids in plant samples (Callado, 2018; Jaren *et al*., 2022; Gloerfelt-Tarp *et al*., 2023) and products (Chen *et al.,* 2021). Raw material variability of cannabis raw materials can affect the quality of final products. Considering the diverse chemical compositions and disadvantages of chromatographic techniques. The objective of this study was to develop the non-destructive estimation of THC and CBD in cannabis extracts using NIR for fast and inexpensive measurement.

Materials and methods

Sample preparation

Cannabis sativa L. is grown in multiple locations with different cultivation techniques including cultivation substrates and pre-harvest treatments. Inflorescences of cannabis plants were harvested and air-dried at room temperature in the dark until reaching 10 % moisture content. All leaves were removed, and the samples were placed in an oven at 40 ºC until they reached constant weight. Stems were then removed from the inflorescence. Flowers were ground and 0.3 g of each sample were weighed. Ground samples were extracted using 10 mL of 95% ethanol in an ultrasonic bath at 40 ºC for 30 minutes for 3 times. Extracts were filtered using Whatman no.1 filter.

HPLC analysis

Cannabinoid concentrations including CBD, d9-THC and THCA were analyzed using a Hewlett Packard Series 1100 High Performance Liquid Chromatograph (Agilent Technologies Inc., USA) equipped with a UV detector at 220 nm. A Cosmosil column (4.6 × 150 mm) was used for separation. The mobile phase A was 0.1% phosphoric acid in water, while mobile phase B was 0.1% phosphoric acid in acetronitrile. The mobile phase flow rate was 1.5 mL/min and used as a gradient according to Table 1. Standard solutions were prepared using CBD, d9-THC and THCA standards (Cayman Chemical, USA)

to cover the expected concentration range of cannabis extracts. Standards were diluted in isopropyl alcohol and filtered through 0.2 mM nylon syringe filters. Cannabis extract samples were diluted in 95% ethanol and filtered using 0.2 mM nylon syringe filters prior to HPLC analysis.

Table 1. Composition of mobile phase mobile phase

Time (min)	Mobile phase A	Mobile phase B
0	28	72
9	28	72
11	5	95
12	5	95
13	28	72
20	28	72

NIRs analysis

NIR spectra in the wavelength range of 1600 nm to 2400 nm of each sample were measured using an microPHAZIR RX NIR spectroscopy (Thermo Scientific, USA). A total of 100 mL of each Cannabis extract sample was transferred into a 200 mL glass insert and put into an adaptor connected to the microPHAZIR RX. Each sample was replicated 5 times.

THC and CBD concentrations from HPLC analysis and normalized spectra of the same samples were used to develop calibration models for prediction of THC and CBD using Partial least square (PLS) method. These prediction models were then used to predict THC and CBD concentrations of 70 cannabis extract samples.

Results

NIR prediction of THC

HPLC analysis showed distribution of THC concentrations from 7 to 20% (Table 2). Samples with various concentrations of THC were selected to develop calibration models. NIR spectra of cannabis extracts with various concentrations of THC in the wavelength range from 1600 and 2400 nm (Figure 1) were processed using the Savitzky-Glory first-derivative filter to remove the baseline drift (Figure 2).

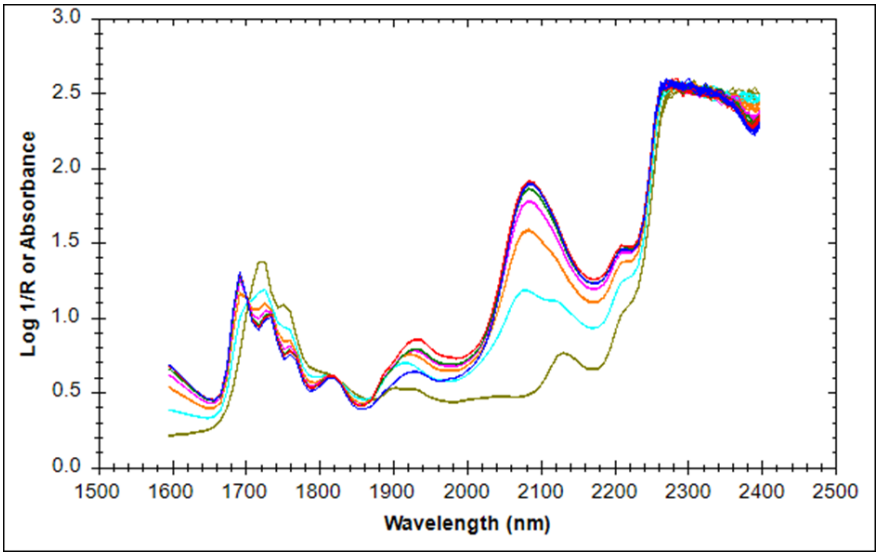

Figure 1. NIR spectra of measured cannabis extracts containing THC at the wavelength range between 1600 and 2400 nm

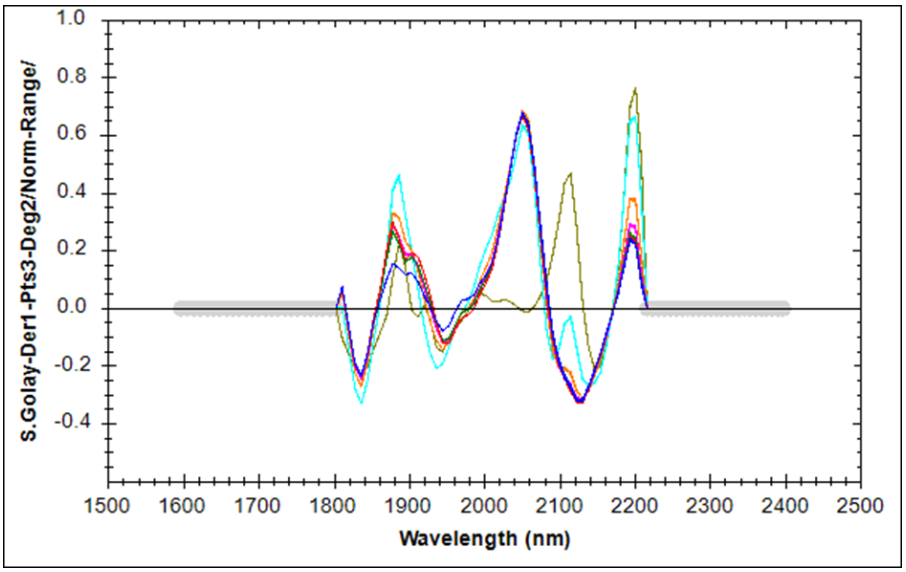

Figure 2. Normalized NIR spectra of measured cannabis extracts after Savitsky-Glory first derivative filter

The PLS calibration plot of the predicted and the actual values of the same samples analyzed by HPLC method was obtained (Figure 3). The calibration plot exhibited good correlations between the predicted and measured THC concentrations in cannabis extract samples with coefficient of determination (R^2)

and root mean square error of calibration (RMSEC) values of 0.9995 and 0.0006, respectively. The PLS model was used to predict THC content in 70 cannabis extract samples. THC concentrations predicted using NIR technique were compared with HPLC method. THC concentrations from the two methods had regression correlation of 0.9078 (Figure 4). Predicted THC concentrations in 70 test samples were in the range of 5.11 to 18.04% (Table 2) which were slightly lower than the actual values obtained from the laboratory analysis using HPLC.

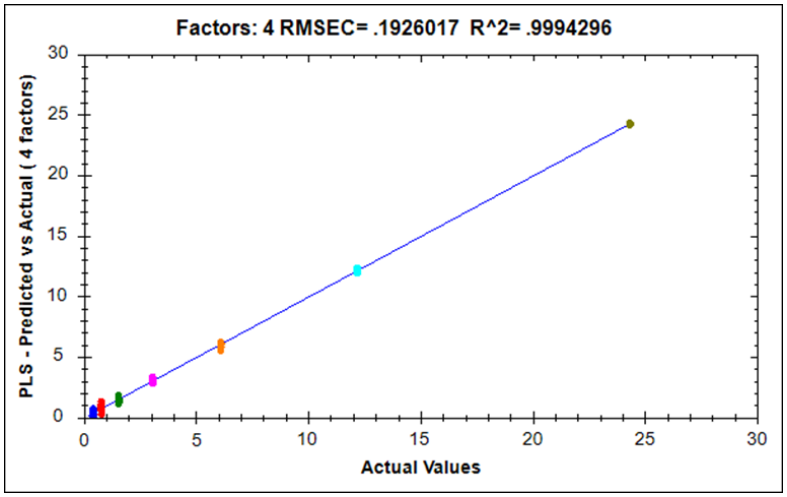

Figure 3. PLS calibration model for THC prediction

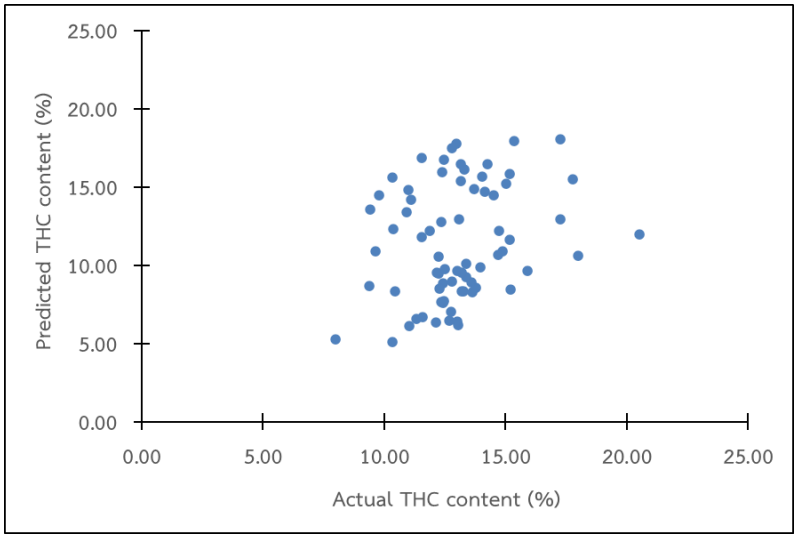

Figure 4. NIR-predicted THC content vs. HPLC analysis results

Table 2. THC concentration (%) in cannabis extract samples using HPLC method and NIR prediction

Sample No.	HPLC	NIR	Sample No.	HPLC	NIR
1	13.37	9.26	36	14.52	14.45
2	14.89	10.86	37	13.59	8.92
3	9.41	13.54	38	11.33	6.58
4	11.09	14.18	39	13.21	8.31
5	13.16	16.43	40	13.76	8.55
6	9.38	8.69	41	12.47	7.71
7	11.54	16.84	42	12.48	9.78
8	15.18	15.81	43	13.15	15.35
9	14.75	12.17	44	12.43	7.62
10	13.08	12.94	45	12.11	6.36
11	10.91	13.41	46	14.02	15.68
12	13.72	14.85	47	17.97	10.59
13	12.40	15.92	48	13.01	9.67
14	13.19	9.51	49	12.77	8.95
15	13.27	8.36	50	11.04	6.10
16	14.69	10.69	51	13.03	6.16
17	13.38	10.08	52	12.35	7.65
18	17.25	12.93	53	12.23	9.45
19	15.03	15.22	54	12.23	10.53
20	12.42	8.85	55	12.18	9.53
21	12.34	12.74	56	13.01	6.43
22	14.14	14.67	57	10.33	5.11
23	10.38	12.30	58	9.63	10.91
24	15.16	11.65	59	7.99	5.24
25	10.43	8.34	60	15.33	17.95
26	12.66	6.47	61	17.77	15.50
27	12.75	7.05	62	13.29	16.12
28	11.57	6.68	63	11.53	11.81
29	13.95	9.86	64	11.85	12.19
30	20.50	11.99	65	10.98	14.79
31	15.91	9.65	66	10.34	15.60
32	15.21	8.46	67	14.25	16.46
33	17.25	18.04	68	12.98	17.79
34	13.62	8.30	69	9.79	14.48
35	12.27	8.53	70	12.45	16.75

NIR prediction of CBD

HPLC analysis showed all samples contain CBD in the range of 0.013 to 0.043 % (Table 3). NIR absorption spectra from pre-selected samples with the lowest and highest CBD concentrations were shown in the figure 5. The absorption spectra were smoothed using the Savitzky-Glory algorithm (Figure 6) to remove artificial noise prior to model calibration.

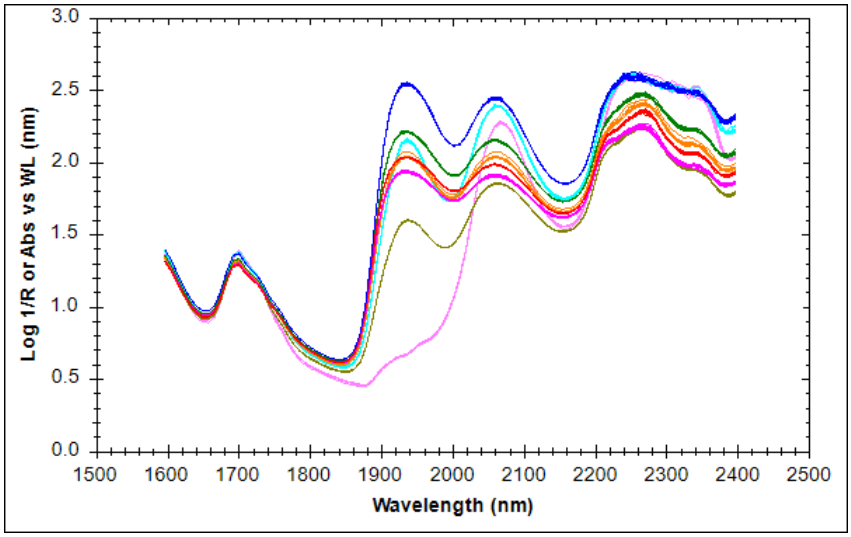

Figure 5. NIR spectra of measured samples containing CBD between 1600 and 2400 nm

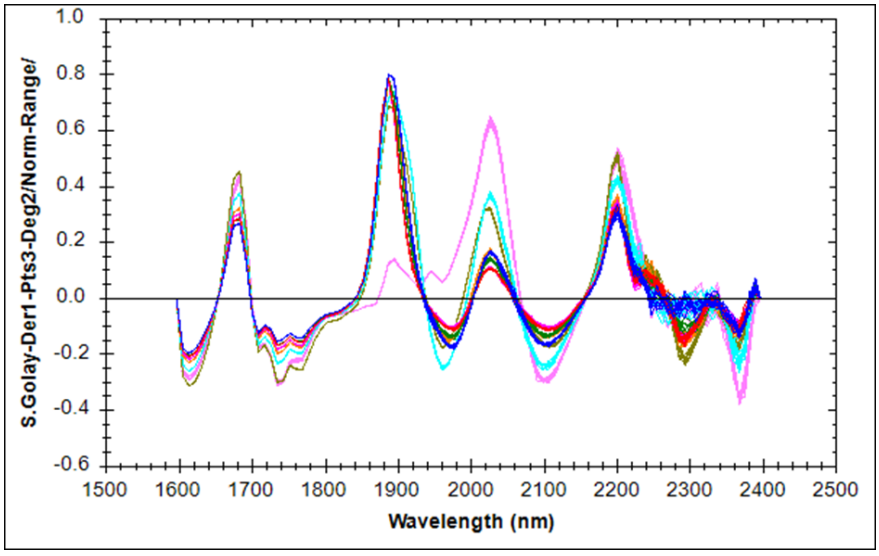

Figure 6. Normalized NIR spectra of measured samples after Savitsky-Glory first derivative filter

The model calibration plot developed by PLS method using processed spectra (Figure 7) showed high correlation with R^2 and RMSEC values of 0.9995 and 0.0006, respectively. This PLS model was used to estimate the power of the model to predict CBD content of 70 cannabis extract samples. The estimated

CBD concentrations obtained from the PLS model were in the range of 0.01 to 0.04% (Table 3) which were close to the actual values measured by HPLC. The correlation between predicted CBD concentrations by NIR and actual CBD content measured by HPLC was relatively high with R^2 of 0.9413 (Figure 8).

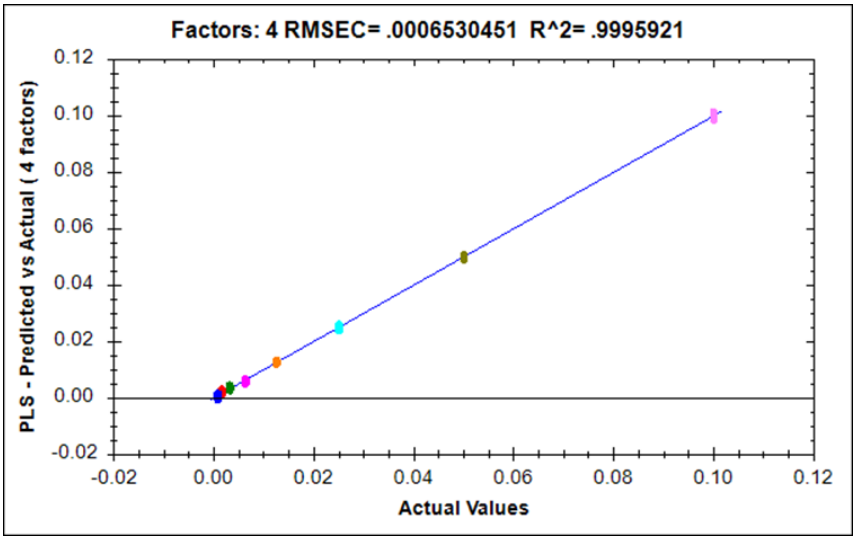

Figure 7. PLS calibration model for CBD prediction

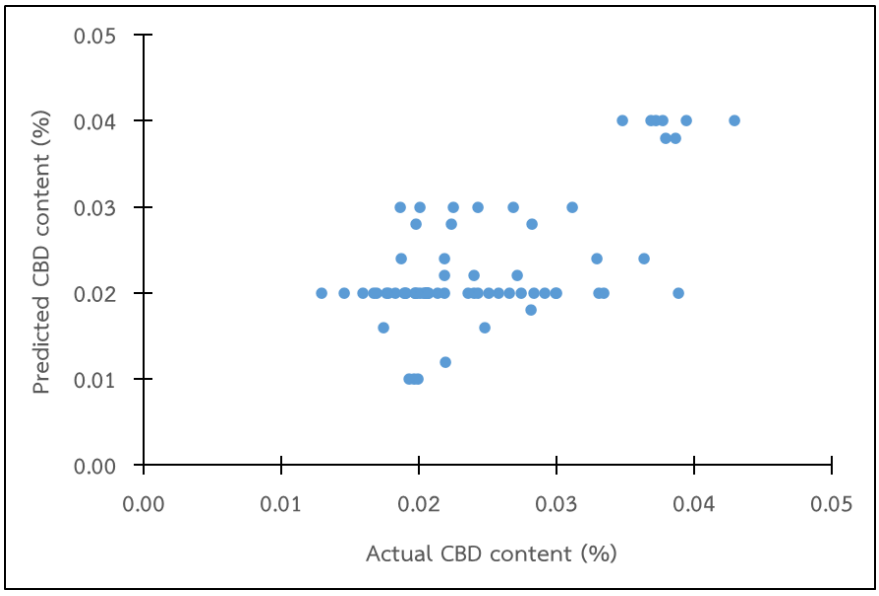

Figure 8. NIR-predicted CBD content vs. HPLC analysis results

Table 3. CBD concentration (%) in cannabis extract samples using HPLC method and NIR prediction

Sample No.	HPLC	NIR	Sample No.	HPLC	NIR
1	0.028	0.020	36	0.021	0.020
2	0.028	0.028	37	0.024	0.020
3	0.018	0.020	38	0.020	0.020
4	0.017	0.020	39	0.017	0.016
5	0.020	0.020	40	0.020	0.020
6	0.016	0.020	41	0.021	0.020
7	0.022	0.012	42	0.021	0.020
8	0.025	0.020	43	0.025	0.016
9	0.013	0.020	44	0.039	0.020
10	0.020	0.020	45	0.019	0.020
11	0.020	0.010	46	0.021	0.020
12	0.024	0.020	47	0.021	0.020
13	0.023	0.030	48	0.019	0.024
14	0.027	0.030	49	0.022	0.024
15	0.020	0.030	50	0.018	0.020
16	0.030	0.020	51	0.020	0.020
17	0.024	0.020	52	0.022	0.028
18	0.027	0.020	53	0.021	0.020
19	0.019	0.030	54	0.020	0.028
20	0.019	0.020	55	0.026	0.020
21	0.020	0.010	56	0.033	0.020
22	0.019	0.020	57	0.019	0.010
23	0.033	0.024	58	0.017	0.020
24	0.015	0.020	59	0.024	0.030
25	0.024	0.022	60	0.038	0.038
26	0.022	0.022	61	0.039	0.040
27	0.028	0.018	62	0.037	0.040
28	0.033	0.020	63	0.039	0.038
29	0.036	0.024	64	0.037	0.040
30	0.027	0.020	65	0.043	0.040
31	0.021	0.020	66	0.030	0.020
32	0.020	0.020	67	0.029	0.020
33	0.022	0.020	68	0.031	0.030
34	0.027	0.022	69	0.035	0.040
35	0.018	0.020	70	0.036	0.040

Discussion

Laboratory measurement of bioactive compounds using standard procedure is more sensitive and accurate but NIR technique for non-destructive measurement is rapid, cost-effective and environmentally friendly with no sample preparation (Cozzolino, 2009). In cannabis plants, THC and CDB are major compounds that normally analyzed using HPLC. However, HPLC analysis

is expensive, time consuming and required sample preparation. In this study, NIR reading was less than a minute per analysis, while the HPLC total run time for each sample was 20 minutes and the retention times for CBD, d9-THC and THCA were 9.3, 13.5 and 14.7 minutes, respectively. Application of NIR measurement for THC and CBD in our study seems to have many advantages over the laboratory method. However, NIR is an indirect measurement as NIR spectra do not directly represent particular molecules, but rather contain information of chemical bonds. Thus, any compounds having the same bonds can potentially be confused (Manley, 2014). The prediction accuracy depends on many factors including wavelength, instrument type, spectral pretreatments, variable selection methods, detection modes and calibration methods (Xu *et al.,* 2019).

The coefficient of determination of THC and CBD calibration models developed using PLS method in this study were 0.9994 and 0.9995, respectively. Prediction models developed from this study showed R^2 greater than 0.98 in both models indicating that these models are accurate and suitable or all applications. However, the R^2 values only show the quality of the calibration not the prediction accuracy. In general, pre-selection of samples used in the calibration model will result in high prediction accuracy (Fan *et al*., 2009) but our population variance was low making the prediction accuracy in this study quite low (R^2 was 0.9078 for THC prediction and 0.9413 for CBD prediction) compared to a previous study in which calibration plots gave good correlations between the predicted and actual CBD measurements with R^2 greater than 0.98 (Chen *et al.*, 2021). In Chen's study, a Fourier-transform infrared spectroscopy (FTIR) was used at wavelength 4500 to 9000 nm for determination of CBD in hemp oil samples while our study used NIR at the wavelength range from 1600 to 2400 nm. In addition, self-optimizing support vector elastic net (SOSVEN) showed lower validation errors than PLS method.

There are many factors affecting calibration models and prediction accuracy. Only PLS method was used to develop calibration model in our study, other regression methods may be further applied to see whether the calibration models are improved as the PLS regression may reach its limits when the data is complex. Machine learning (ML) methods such as artificial neural networks (ANN) and support vector regression (SVR) are alternative methods for complex data sets (Chen *et al.*, 2019; Chen *et al.,* 2021). Increasing the sample size with more variance population for developing calibration model is another factor that can improve the prediction accuracy. A previous study investigated the effect of data size and preprocessing of different product categories including grain, dairy, petfood and compound feed. The results showed that increasing number of

samples for calibration data improved prediction performance (Schoot *et al*., 2020).

Our prediction models are considered moderately successful, but they provide meaningful results which are good for quality assurance. Non-destructive of THC and CBD using NIR is still a promising method for a rapid and low-cost measurement of THC and CBD in cannabis extract and other products. Further work on validating the results using large and diverse samples will improve estimation accuracy.

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