Rapid Quantitative Detection of Cannabinoids using Laser Raman Spectroscopy

Oranat Chuchuen
Department of Chemical Engineering and Biomedical Engineering Program, Faculty of Engineering, Khon Kaen University, Khon Kaen, Thailand | Mekong Health Science Research Institute, Khon Kaen University, Khon Kaen, Thailand
oranat@kku.ac.th

Rungtip Madee
Research and International Relations Affairs, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
rungtma@kku.ac.th

Jakkapat Paluka
Cannabis Research Institute, Khon Kaen University, Khon Kaen, Thailand
jakkapat_pa@hotmail.com

Chanon Lapjit
Department of Horticulture, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand | Cannabis Planting and Extraction Center, Khon Kaen University, Khon Kaen, Thailand
lchano@kku.ac.th

Pewpan M. Intapan
Mekong Health Science Research Institute, Khon Kaen University, Khon Kaen, Thailand
pewpan@kku.ac.th (corresponding author)

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ABSTRACT

The current gold-standard methods for cannabinoid measurement are highly sensitive, yet expensive, sophisticated, and time-consuming. This study investigated the potential of laser Raman spectroscopy as a rapid and straightforward method for the quantitative detection of three major cannabinoids: Cannabidiol (CBD), Cannabinol (CBN), and Delta-9-tetrahydrocannabinol (THC). A series of solutions of the cannabinoids extracted from *Cannabis sativa* were prepared in Tetrahydrofuran (THF) and measured with a Near-Infrared (NIR)-excited Raman microspectrometer. The Raman spectra were subjected to chemometrics-based multivariate analysis, employing an ordinary least square fitting method. A strong linear relationship ($R^2 > 0.98$) was observed between the Raman intensity and the concentration of all studied cannabinoids. A Raman-based prediction model of each cannabinoid was developed based on a leave-one-out cross-validation analysis, which yielded a minimum detectable concentration in the range of 0.23-0.42 mg/ml, as determined by the Root Mean Square Error of Cross Validation (RMSECV). Additionally, characteristic Raman marker bands for each cannabinoid were identified. Overall, this study presented a simple yet effective Raman spectroscopy-based technique for rapid label-free cannabinoid detection and measurement.

Keywords-cannabidiol; cannabinol; delta-9-tetrahydrocannabinol; Raman spectroscopy; optical spectrometry

I. INTRODUCTION

The application of cannabinoids and cannabis-containing products for medical purposes has been on the rise and has been the subject of extensive research for several decades. Cannabinoids are extracted from the Cannabis genus, which comprises three major species: *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis* [1]. The concentration of cannabinoids varies among these species. The two most commonly used cannabinoids for medical applications are THC

and CBD, with the latter being a non-toxic compound derived from cannabis, which has shown therapeutic benefits in the treatment of chronic pain [2] and psychiatric disorders [3]. While THC has demonstrated certain therapeutic benefits, excessive use may result in adverse cognitive effects [4], cardiovascular issues, and anxiogenic effects [5]. Additionally, there is a potential for postnatal impaired cardiac function [6]. The concentration of these cannabinoids varies depending on several factors, including the species of the cannabis plant and the extraction method used. It is therefore imperative to ascertain the concentrations of these cannabinoids in each extracted batch, in order to ensure the effective and safe medical use of the product.

The current gold-standard, highly sensitive method for cannabinoid measurement is High-Performance Liquid Chromatography (HPLC). However, this method is expensive, time-consuming, complex, and requires experienced technicians. Accordingly, there is a growing need for a straightforward, cost-effective method for quantitative analysis of cannabinoids. Spectroscopic techniques are typically suitable for rapid and non-destructive chemical identification and have demonstrated potential in a diverse array of applications, including food and agricultural product quality monitoring and contamination detection [7, 8]. Raman Spectroscopy (RS) is a laser-based optical spectrometry technique that enables the non-invasive detection and quantitative measurement of analytes. In Raman scattering, the shift in the energy of scattered photons produces discrete lines, known as Raman shifts, which correspond to the spectral signatures (i.e., fingerprints) of the molecules in question. RS has been proven effective in a multitude of applications, including forensic science [9], material characterization [10], and plant research [11]. Raman spectroscopy has demonstrated considerable potential for use in biomedical research and medical diagnosis [12, 13]. It facilitated rapid and non-invasive detection of small molecules and disease metabolites in biological fluids and specimens [14, 15]. Furthermore, it has been effectively employed for the quantitative analysis of pharmaceuticals in a range of relevant matrices and tissues, as evidenced by several studies [16-19]. Recently, Raman spectroscopy has been employed as an analytical tool in the field of cannabis research. Surface-Enhanced Raman Scattering (SERS), which is a Raman signal enhancement approach based on the use of metal nanoparticles, has been the subject of investigation for the characterization and quantitative detection of cannabinoid compounds [20-22]. However, in comparison to conventional RS, the SERS approach is more intricate and expensive, necessitating the fabrication of SERS substrates or chips. Recently, conventional RS has been employed for the spectral differentiation of various types of hemp and cannabis plants [23-25] and the spectroscopic characterization of major cannabinoid compounds [26]. Nevertheless, the capacity of conventional label-free RS to perform a quantitative analysis of the three principal cannabinoids (CBD, THC, and CBN) has yet to be fully elucidated. This study examined the potential of RS for a rapid, marker-free measurement of the three cannabinoid compounds. The characteristic Raman features of each compound were documented, along with their vibrational assignment, quantitative analysis, and detection sensitivity.

II. MATERIALS AND METHODS

A. Cannabinoid Sample Preparation

Cannabinoid compounds, including CBD, THC, and CBN, were prepared from *Cannabis sativa* KKU01, which was cultivated in an indoor system at Khon Kaen University, Thailand. The female flowers of KKU01-*Cannabis sativa* were subjected to a further ethanol extraction process following the drying of the flowers [27]. The solvent from the crude extract sample was evaporated under reduced pressure using a Rotavapor R-300 (Buchi, Switzerland). The crude extract sample was subjected to normal-phase flash column chromatography, after which it was further purified by preparative high-performance chromatography (prep-HPLC) (Pure C-810 flash, Buchi, Switzerland) with a reverse-phase cartridge column (FlashPure ID C18 40 g; 40 µg, Buchi, Switzerland). The isolated cannabinoids were subjected to analysis by Nuclear Magnetic Resonance (NMR) (Ascend-400, Bruker, Germany) and HPLC (Alliance HPLC System, Waters, USA). Subsequently, stock solutions of CBD, THC, and CBN were dissolved and serially diluted with THF to prepare cannabinoid solutions of varying concentrations in the range of 0.1–10 mg/ml.

B. Raman Measurement

To perform Raman measurements, each cannabinoid sample (14 µl) was dropped onto a hole-drilled stainless steel well plate. Subsequently, a quartz coverslip (R52500, Esco Optics, Oak Ridge, NJ) was placed over the sample well to prevent solvent evaporation. The samples were analyzed using a Horiba XploRA PLUS confocal Raman microscope (Horiba Jobin Yvon, Northampton, UK) with a 50x objective lens (LMPLFL50X, Olympus, St. Joseph, MI) and an excitation laser of 785 nm wavelength. Each sample was measured three times independently, with an integration time of 240 seconds, and its Raman spectra were recorded using LabSpec 6 software (Horiba Scientific, Edison, NJ).

C. Raman Spectral Processing and Quantitative Analysis

The acquired Raman spectra were subjected to spectral processing in MATLAB (MathWorks, Natick, MA). The Raman spectra were cropped to a range of 200-1900 cm^{-1}, baseline-subtracted with a fourth-order polynomial function, smoothed with a Savitzky-Golay filter of nine smoothing points, and normalized with the mean spectral intensity of all wavenumbers, unless otherwise stated. To obtain a Raman spectrum of each cannabinoid, the spectrum of the cannabinoid solution in THF was subtracted from the THF spectrum obtained with the same acquisition time. For the purposes of quantitative analysis, all Raman spectra were fitted using the classical least squares method, with the pure spectral components of each cannabinoid compound and THF as the variables. The ratio of the spectral contribution (i.e., the integrated area under all spectral peaks) of each cannabinoid to that of THF for each serially diluted concentration was determined. A leave-one-out cross-validation approach was employed to obtain linear regression models for Raman-predicted concentrations. The Limit of Detection (LOD) was estimated from the root mean squared error of cross-validation

RMSECV, which represents the accuracy of the Raman-based models for cannabinoid concentration measurement.

III. RESULTS AND DISCUSSION

Each cannabinoid exhibited distinctive Raman spectral signatures within the fingerprint region (300-1,900 cm^{-1}), which could be employed for Raman detection and identification, as shown in Table I and Figure 1. The most evident region for discerning the spectral dissimilarities between the three compounds was observed to be within the Raman shift range of 1,500-1,700 cm^{-1}.

TABLE I. TENTATIVE VIBRATION ASSIGNMENT OF EACH CANNABINOID'S SALIENT RAMAN BANDS

Cannabinoid	Raman peak (cm-1)	Moiety	Band assignment	Ref.
CBD	550	Isopropenyl group	ν(C-C); γ,δ(C-H)	[28]
	774	Cyclohexene ring	ρ(CH2)	[28]
	1435	Alkyl chain	ε(CH2)	[28]
	1621	Benzene ring	ν(C-C)	[23, 28]
	1644	Isopropenyl group	ν(C=C)	[28]
	1665	Cyclohexene ring	ν(C=C)	[28]
THC	547	Benzene ring	Valence angle bend	[28]
	788	Cyclohexene ring	γ,δ(C-H)	[28]
	1437	Cyclohexene ring	ε(CH2)	[28, 29]
	1623	Benzene ring	ν(C-C)	[23, 28]
	1666	Cyclohexene ring	ν(C=C)	[28]
CBN	705	Benzene rings	ν(C-C)	[30]
	1305	Benzene rings	ν(C-C); β,δ(C-H)	[30]
		Alkyl chain	τ(CH2)	
	1443	Alkyl chain	ε(CH2)	[28, 31]
	1506	Benzene rings	ν(C=C); β,δ(C-H)	[30]
		Pyran ring	τ(CH3)	
	1622	Benzene rings	ν(C=C); δ(C-H)	[30]

ν: stretching; β: in-plane; γ: out-of-plane; δ: bending; ε: scissoring; ρ: rocking; τ: twisting

All three cannabinoids exhibited discrete Raman bands at 1,621-1,623 cm^{-1}, which were attributed to the vibrations in the benzene rings [23, 28, 30]. However, CBD exhibited a distinctive Raman peak at 1,644 cm^{-1}, which was not evident in the Raman spectra of THC and CBN. This peak may be attributed to the C=C stretching vibration of the isopropenyl group [28], which was absent in THC and CBN. Furthermore, CBD and THC displayed a Raman peak at approximately 1,665 cm^{-1}, which was attributed to the C=C stretching vibration of the cyclohexene ring. This structural moiety was not present in CBN. CBN exhibited a distinctive Raman band at 1,506 cm^{-1}, which was attributed to the vibrations in the benzene and tetrahydropyran rings [30]. Moreover, CBN displayed a markedly intense Raman band at 1,305 cm^{-1}, predominantly ascribed to the vibrations of the two benzene rings intrinsic to its structure. The three cannabinoid compounds demonstrated Raman bands at 1,435-1,443 cm^{-1}, which could be attributed to the CH_2 rocking vibration of the alkyl chains [28, 31] and the cyclohexene rings [28, 29]. Additionally, CBD and THC exhibited a notable Raman band attributed to cyclohexene ring vibrations at 774 cm^{-1} and 788 cm^{-1}, respectively [28]. This Raman feature was absent in CBN due to the absence of the cyclohexene ring. Another prominent band of CBD and THC was also observed at approximately 550 cm^{-1}, resulting from the vibrations of the isopropenyl group and the benzene ring, respectively. Consequently, each cannabinoid exhibited distinctive Raman peaks that could be effectively utilized as marker bands for their spectral identification.

It is crucial to highlight that the solvent used in this investigation was tetrahydrofuran, a hydrocarbon compound that exhibits a comparatively elevated Raman scattering cross-section in comparison to the analytes. Therefore, the cannabinoid Raman peaks may have been masked in regions where the solvent peaks were prominent [32]. To address this challenge, the Raman spectra of the cannabinoid solution were subjected to analysis using a chemometrics method based on the multivariate classical least-squares fitting approach, as illustrated in Figure 2.

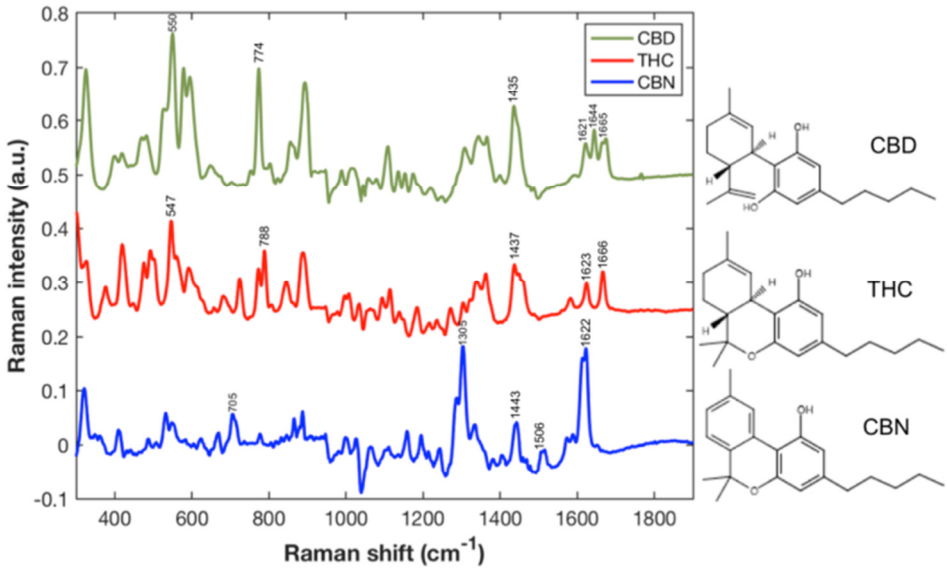

Fig. 1. Raman spectra of CBD, THC, and CBN. All spectra of cannabinoid solution in THF were normalized by the maximum intensity of the THF's Raman peak at 1,030 cm^{-1} before being subtracted by a THF spectrum. The spectra were vertically offset for display clarity.

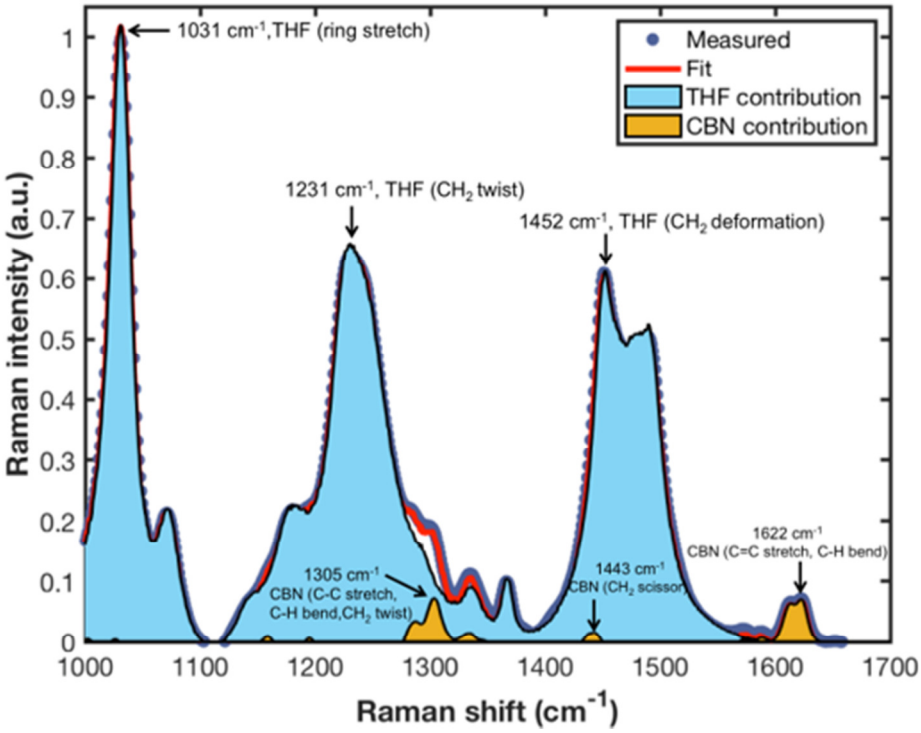

Fig. 2. A representative ordinary least-squares fit approach applied to a CBN solution spectrum, which was spectrally decomposed to determine the underlying spectral contribution of each basis component.

Each Raman spectrum was fitted with pure spectral components of the studied cannabinoid and the solvent THF in order to determine the underlying spectral contribution of each basis component. The ratio of the spectral contribution of the cannabinoid compound to that of the solvent was related to the known concentration of the compound in order to establish a standard curve for each studied compound. This approach employed the spectral data from the entire Raman spectrum for quantitative analysis, rather than relying on a single Raman peak that could have been influenced by the vibrational bands of the solvent. Therefore, this method is more reliable and has been validated for the quantitative analysis of the Raman spectral data of pharmaceutical and biological molecules in previous studies [17, 18]. Despite the presence of prominent bands of THF in the Raman spectra, distinct peaks for each cannabinoid were still discernible (Figure 3 (a), (c), and (e), highlighted in gray).

As the concentration of the cannabinoid in question decreased, the size of the characteristic peaks in question also diminished. The spectral features of each cannabinoid were extracted from the Raman spectra using the chemometrics-based approach previously discussed. The Raman signal intensities exhibited a strong linear concentration dependence ($R^2 > 0.98$) for all cannabinoids across the entire range of concentrations, which is consistent with the principles of spontaneous Raman scattering. In other words, the Raman emission signal increased in a linear fashion with concentration within the focal volume, indicating the potential of Raman spectroscopy for precise quantitative analysis of cannabinoids.

Figure 4 shows the cross-validated, Raman-based predictions for each cannabinoid. The regression models demonstrated no significant deviation from linearity, with slopes not differing from 1 ($p > 0.05$, ANOVA) for all cannabinoids. This finding indicated that the Raman-based approach provided a reliable estimate of the true cannabinoid concentrations. The LOD was estimated from the RMSECV, which has been validated to provide a reliable estimate of the minimum detectable concentration of analytes [33], and was determined within the range of 0.02-0.04% w/w. In comparison, it was reported that the THC content in a cannabinoid resin excreted from the flowers of female cannabis plants ranged between 0.5% and 7% [34]. Furthermore, the European Union (EU) has established a legal limit of 0.2% THC content for industrial hemp crops [35], while the United States and Canada have set the limit at 0.3% [36]. Therefore, the conventional RS-based approach is sufficiently sensitive to detect physiologically and legally relevant cannabinoid concentrations, indicating its potential for practical use in quantitatively detecting cannabinoid compounds. However, increasing the acquisition time could further enhance the prediction accuracy and detection limit, as the minimum detectable concentration is demonstrated to be directly proportional to the inverse square root of the integration time [37].

IV. CONCLUSIONS

As the use of medical cannabis has increased, there has been a corresponding rise in demand for a rapid, straightforward, yet accurate method of quantifying

cannabinoids. The current gold-standard technique for cannabinoid measurement is sophisticated, costly, time-consuming, and labor-intensive, which could present a challenge for implementation, especially in regions with limited resources. While Raman Spectroscopy (RS) has been employed qualitatively for the characterization and differentiation of cannabis, the quantitative detection of cannabinoid compounds has been predominantly investigated using the Surface-Enhanced Raman Scattering (SERS) approach. Despite its capacity to detect analytes at ultra-low concentrations, SERS is a more costly technique than conventional RS. This is due to the necessity of utilizing a nanoparticle-based substrate for signal enhancement, which frequently requires intricate preparation and fabrication processes. Conventional substrate-free RS is a more straightforward technique, but it is less sensitive. However, its potential for a quantitative analysis of the three major cannabinoid compounds, Cannabidiol (CBD), Cannabinol (CBN), and Delta-9-tetrahydrocannabinol (THC), has yet to be fully realized.

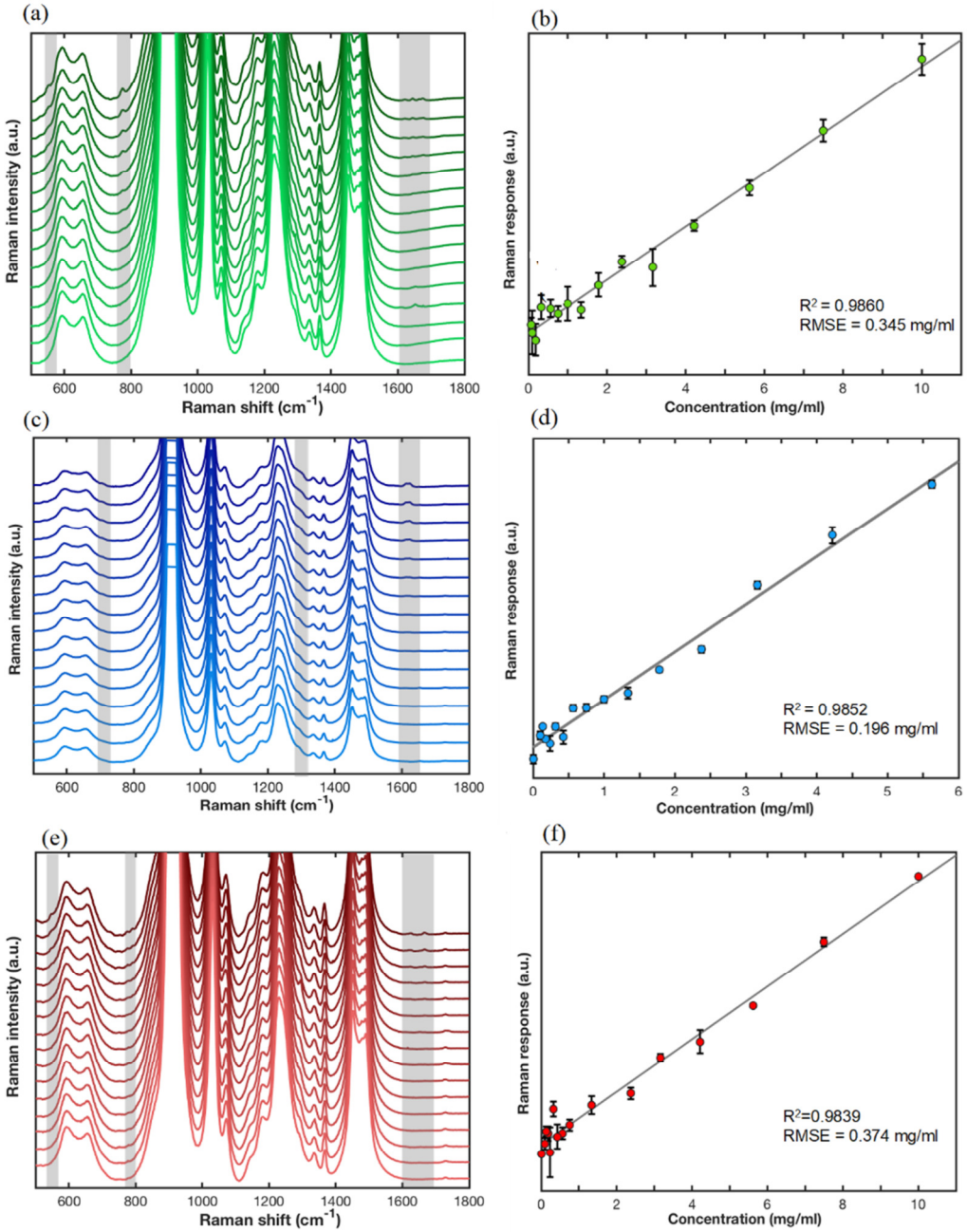

Fig. 3. Concentration-dependent Raman spectra of CBD (a), CBN (c), and THC (e) along with their standard curves of normalized cannabinoid Raman signal vs. concentration (b), (d), and (f). For presentation clarity, the Raman spectra were vertically offset, with decreasing cannabinoid concentrations from top to bottom. Each data point in the standard curves represents the mean ± SE of three independent measurements.

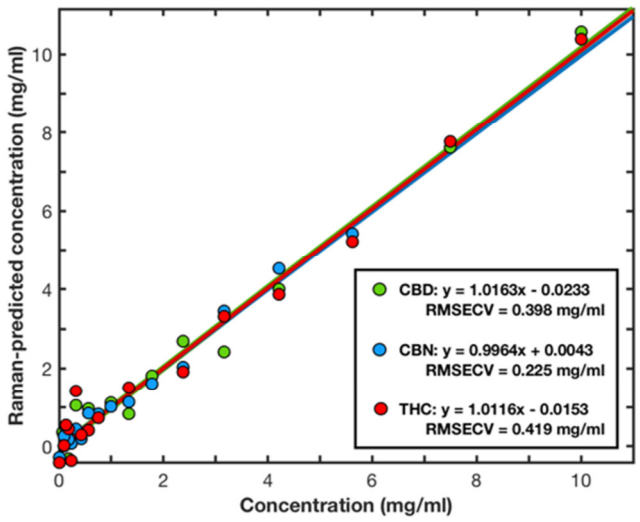

Fig. 4. Cross-validated, Raman-based predictions of CBD, CBN, and THC concentrations.

This research developed a technique based on conventional RS for the rapid quantitative analysis of three major cannabinoids. All of the cannabinoids under investigation displayed distinctive Raman characteristics that could be employed for their qualitative identification. The study demonstrated that Raman spectrometry has the potential to quantitatively analyze cannabinoids, as evidenced by the strong linear correlations observed between the Raman signal intensities and the concentrations of the cannabinoids. A Raman-based prediction model was developed for each cannabinoid and cross-validated using the leave-one-out cross-validation approach. The minimum detectable concentration, as determined by the Root Mean Square Error of Cross Validation (RMSECV), was found to be approximately 0.2 to 0.4 mg/ml, which is relatively lower than the practical level of cannabinoid concentration. While the SERS technique may provide a detection limit that is substantially lower than this level, the conventional RS approach used in this study achieved a sufficiently low detection limit that is practically relevant. Furthermore, the approach has the added advantage of being substrate- and label-free, which makes it easier to implement without the need for additional sophisticated Raman-enhancing metallic nanoparticle materials. In conclusion, the findings of this study demonstrate that conventional RS is a promising and reliable technique for the simple, rapid, reagent-free, and non-destructive detection and measurement of cannabinoids in a cost-effective manner, rendering it an appropriate choice for implementation even in resource-limited settings.

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