Analysis of THC and CBD in Cannabis Commercial Oil: Preliminary Study

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ABSTRACT

Currently, cannabis oil is widely used without any control over production quality. It is therefore concerned that the unregulated number of active ingredients contained in the oil may affect the Thai public health system. To analyze the number of active ingredients in cannabis oil available on the market, this study was conducted. Δ9-THC and CBD quantification were analyzed with UPLC-PDA, linearity, LOD, and LOQ validity study. The method was then analyzed for Δ9-THC and CBD content in 60 cannabis oil samples. In this study, the method was linear in the concentration ranges of 0.50-5.00 μg/mL, with a correlation coefficient of >0.99 for all samples. The sensitivity was excellent, with LOD and LOQ of 0.10 μg/mL and 0.50 μg/mL of both. The accuracy and precision were very well with % recovery and % RSD of 95.85-106.44 and 1.68-6.54 for Δ9-THC, 88.58-102.21, and 0.67-5.97 for CBD, respectively. Δ9-THC and CBD content in cannabis oil was found. Each sample varies greatly, ranging from 0.00 -1,225.25 μg/mL for Δ9-THC, and 0.00-524.47 μg/mL for CBD. Therefore, the patient's use of cannabis oil with an unknown amount of the active ingredient may affect the health of the user.

Keywords: Δ9-THC and CBD, Cannabis oil, UPLC-PDA

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Introduction

The Narcotics Act (No. 7), Buddhist era 2562 classifies marijuana as a category 5 narcotic but allows the use of marijuana only for medical purposes. At present, various information available about the benefits and harms of using marijuana in Thai people is, therefore, few and relatively new. Use in certain conditions for some diseases there is insufficient evidence for a decision to use medical marijuana. On the other hand, there are still voices from many medical marijuana users that it is good and useful. From the preliminary information, it causes confusion for medical personnel and the general public. Therefore, it is a good opportunity for the researcher to collect empirical evidence to confirm the quality. Of commercially available cannabis oils (by taking 60 samples of cannabis oil studied to represent non-manufactured cannabis oil from a certified medical company) Including basic indications make a decision to use medical marijuana that is truly beneficial for medical personnel to continue treating patients. Both now and in the future.

In 2019, the Ministry of Public Health of Thailand distributed cannabis oil that has been prepared with production standards and prepared by the Government Pharmaceutical Organization (GPO) to hospitals participating in the medical cannabis oil trial project. The production of the organization has a certain proportion and quantity of active ingredients that can be controlled, it is similar to those used abroad. GPO has produced cannabis oil in three trials, including a formula that mixes Δ9-THC: CBD 1:1 with Δ9-THC of 27 mg/ml and CBD of 25 mg/ml, the Δ9-THC-only formulation contained 0.5 mg/ drop of Δ9-THC, and the CBD-only formulation contained 100 mg/ml of CBD. These are known concentrations and controlled for use by physicians trained in the therapeutic use of cannabis, but all of these are manufactured for experimental purposes only.

The cannabis plant (Cannabaceae) in each species contains a different amount of psychoactive compound [1-2]. Cannabis sativa and cannabis indicia are popular cannabis plants used for the extraction of delta-9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD), which both have different proportions of active ingredients, C. Sativa contains less Δ9-THC and CBD and C. indica contains more Δ9-THC and CBD and has many other substances [3]. Therefore, the production of cannabis oil in Thailand which does not have a quality control system depends only on folk wisdom or folk medicine with different production formulas and may be very different. However, there have not been any systematic studies reporting on the concentrations of each formulation and the proportion of Δ9-THC and CBD. Δ9-THC has no definitive research data that can cure disease, especially in Thailand. There is only belief, but there have been studies showing Δ9-THC is effective in treating disease conditions in experiments [4-6]. Even so, Δ9-THC has been reported to have a greater effect on the body than cure admitted in hospitalized patients [7], especially in patients who self-dose cannabis oil and experience dizziness and vomiting. In 2020, the US Food and Drug Administration (FDA) approved CBD as a potential treatment for seizures in children [8] and used as a treatment for these conditions, but in Thailand, there is no

certification, and it is unalloyed to produce in general. However, it has been found that widely available products, especially cannabis oil, for which the proportion of the active ingredients in the product is unknown. Therefore, the researcher would like to study the number of active ingredients and the proportion of Δ9-THC and CBD in commercial cannabis oil.

Materials and Methods

Instruments and Analytical Conditions

Acquity™ Ultra Performance Liquid Chromatography (Waters Corporation, Milford, MA, USA) was used for the separation module. Chromatographic separation was developed on an ACQUITY UPLC™ HSS T3 column (1.8 μm, 100 mm x 2.1 mm I.D.) (Waters Corporation, Milford, MA, USA). To achieve an optimum result, the mobile phase was performed with a flow rate of 0.45 mL/min and a column temperature of 40°C. The gradient programmed for the mobile phase was optimized using acetonitrile and 0.05% formic acid in water. ACQUITY UPLC® Photodiode Array (PDA) Detector (Waters Corporation, Milford, MA, USA) was operated at wavelengths between 200-380 nm and quantitation was optimized on the requirement for high sensitivity and specificity. The auto-sampler was conditioned at 25°C and the injection volume was full loop with 10 μl. Empower 2 software (Waters Corporation, Milford, MA, USA) was used for data management

Chemicals and Reagents

Delta-9 tetrahydrocannabinol (Δ9-THC, THC), cannabidiol (CBD), and delta-9 tetrahydrocannabinol-D3 (THC-D3) (internal standard, IS) were purchased from Sigma-Aldrich Ltd. (Steinheim, Germany). HPLC-grade acetonitrile, methanol, acetone, and formic acid were purchased from Labscan Ltd. (Bangkok, Thailand). Water used for experimentation was produced by the Milli-Q® water purification system (EMD Millipore, Billerica, MA, USA). Any other chemicals used were of analytical grade from Thermo Fisher Scientific (Waltham, MA, USA).

Standard Solution Preparation and Calibration Curve

Delta-9tetrahydrocannabinol (Δ9-THC, THC, cannabidiol (CBD, and delta-9 tetrahydrocannabinol-D_3 (THC-D_3) standards were accurately weighed and then prepared in acetone to stock solutions. Subsequently, primary standard solutions of both were prepared by dilution of stock standard solution with acetone to 1,000 μg/ml. After that, working standard solutions were prepared by dilution of primary standard solutions with acetone to a concentration range of 10.00 – 50.00 μg/ml for THC, CBD, and 10.00 μg/ml for THC-D3. Finally, the six different concentration levels of calibration standards (CS) were prepared by spiking working standard solutions with coconut oil to the final concentration, which was detailed as 0.50, 1.00, 2.00, 3.00, 4.00, and 5.00 μg/ml. The QC samples were prepared separately in the same way to create low, medium, and high controls at 1.00, 2.00, and 5.00 μg/ml, respectively. All

standard stock solutions, primary standard solutions, working solutions, and QC samples were stored at -20°C until use.

Sample Preparation

Fifty μl of IS (10.00 μg/ml) was added into 50 μl of samples in a 1.5mL micro-centrifuge tube and mixed immediately. Then, 100 μl of samples were diluted with 50 μl of acetone and 50 μl of isopropyl alcohol, and mixed immediately. The samples were centrifuged at 14,000 rpm for 10 minutes. All supernatant was transferred into the vial and injected into the UPLC system.

Method Validation

The method validation was carried out at the Food Safety Laboratory, Department of Food, and Aceh Province. The validation stage is carried out based on the SANTE guidelines. [9] The validation parameters tested were selectivity, linearity, LOD, LOQ, precision, and accuracy.

Samples

Samples used in this study were obtained from there are two sources of cannabis oil (sample) used in this study.

1. Obtained from ordering cannabis oil that is available in the market by yourself for study during the year 2020-2021.

2. A total of 60 samples were sent from the patient's physician who was admitted to Siriraj Hospital for analysis at the Clinical Toxicology Laboratory during the year 2020-2021.

Analytical samples from both sources total 60 samples.

Result and Discussion

Selectivity, Linearity, Calibration Curve, LOQ and LOD

The selectivity of the method is determined by comparing the spiked chromatogram with the blank chromatogram. The spiked sample was an analytical sample added with standard Δ^9- THC and CBD, while the blank was not added with the standards. There were only two peaks representing that appeared in the spiked chromatogram. Besides, the blank chromatogram did not show the same retention peaks as the standards' peaks, as shown in figure 1.

The linearity test was performed using the standard concentration series of 0.50, 1.00, 2.00, 3.00, 4.00, and 5.00 μg/ml. The calibration standard curve was constructed by plotting Δ9-THC and CBD peak area. This method also showed good linearity, with a coefficient of determination greater than 0.98 [10-11] as shown in Table 1.

LOD is the minimum concentration of analysis that can be detected, while LOQ is the minimum concentration of analysis that can be measured by the instrument. LOD and LOQ were defined as the signal-to-noise ratio of 2:1-3:1 for LOD and 10:1 for LOQ [12]. This method showed high sensitivity with LOD of 0.10 μg/mL for CBD, 0.12 μg/ml for Δ^9-THC, and LOQ of 0.50 μg/ml for both, showed in Table 1.

Table 1 Validation parameters of linearity, LOD, and LOQ.

Standards	Linearity analysis (μg/mL)	correlation coefficient value	LOD (%CV)	LOQ (%CV)
Δ^9-THC (n=6)	0.50 - 5.00	0.998	0.12 (1.54)	0.50 (2.83)
CBD (n=6)	0.50 - 5.00	0.999	0.10 (0.95)	0.50 (0.84)

Accuracy and Precision

Accuracy and precision were determined based on % recovery and percentage of relative standard deviation (%RSD) through standard measurements with various concentrations of 1.00, 2.00, and 5.00 μg / ml in 6 repetitions. The results showed % recovery and %RSD of 95.85-106.44 and 1.68-6.54 for Δ^9-THC, 87.92-102.21, and 0.67-5.97 for CBD, respectively. This showed the accuracy and precision of measurement had met the requirements by SANTE [9, 13], Δ^9-THC and CBD analysis methods are applicable if % recovery and %RSD are in the range of 70-120% and ≤ 20%, respectively. [14] shown in Table 2.

Table 2 Validation parameters of accuracy and precision.

Standards	**Spike Concentration (μg/mL)**	**Recovery (%)**	**RSD (%)**
Δ^9-THC (n=6)	1.00	95.85±4.65	6.54
	2.00	106.44±8.96	4.54
	5.00	100.92±11.54	1.68
	1.00	102.21±6.36	5.97
	2.00	87.92±5.72	2.85
	5.00	88.58±4.68	0.67

Result

Samples analysis

Analysis of Δ9-THC and CBD concentrations in 60 samples of cannabis oil. This study was Found that the concentrations of Δ9-THC and CBD in each sample were very different, but in some samples, they were similar and in some samples, there were no Δ9-THC and CBD in the Samples (shown in Table 3). This could be because these samples of cannabis oil were improperly produced, with no control, and arise from unknown sources.

Table 3 The concentration of delta-9-tetrahydrocannabinol in commercial cannabis oil

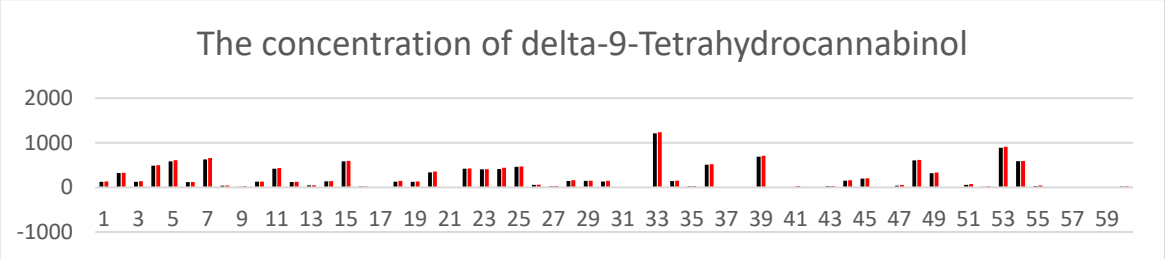


Table 4 The concentration of cannabidiol in commercial cannabis oil

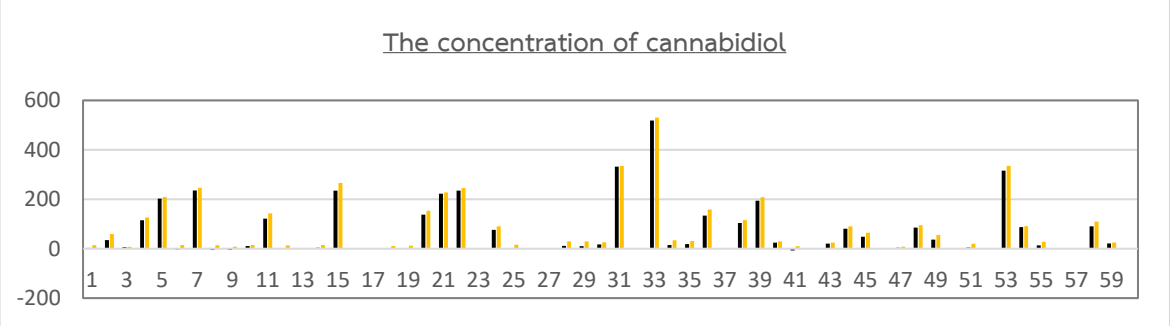
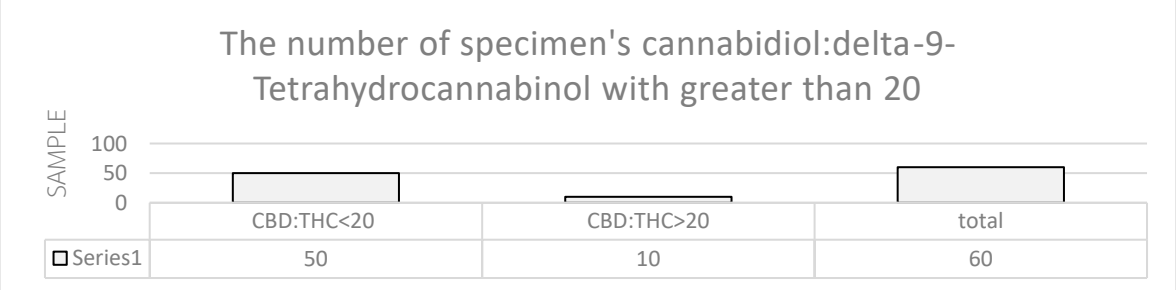


Table 5 The ratio between the number of extracts that contain at least 20:1 more CBD than Δ-9-THC in commercial cannabis oil.



Discussion

All 60 products were submitted for analysis without labeling the active ingredients, so verifying each product's ingredients and quality was impossible. There is no data on the use of oil extracts comparable to use.

1. A cannabis extract with a high content of cannabidiol (CBD) without the use of difficult-to-treat epilepsy. The CBD: THC ratio should be more than 20:1 data from clinical trials of epidiolex® (CBD cannabis oil)

Medical marijuana products [15-16]

1. Marijuana extract is produced by the Government Pharmaceutical Organization.

1.1 THC enriched (13 mg/ml), 1 drop contains 0.5 mg THC

Initiate 1 drop per day if no adverse events occur and the patient does not respond to treatment. The dose can be titrated slowly up to 2 mg per day. (Referring to the use of dronabinol) or at the discretion of the physician. The information that the Department of Medicine has used the dominant THC marijuana extract of the Government Pharmaceutical Organization in patients with End-stage cancer to improve the quality of life of 63 people found that the use THC. Outstandingly effective at an average dosage of 1 mg per day (maximum dosage of 5 mg per day) [17]

1.2 THC: CBD = 1:1 where 1 ml contains 27 mg of THC and 25 mg of CBD or 1 drop contains 1 mg of THC and 1 mg of CBD Initiate 1 drop per day if no adverse events occur and the patient does not respond to treatment. The dose can be adjusted slowly. The maximum recommended dose of THC is no more than 30 mg per day (based on the use of nabiximol)

1.3 CBD enrich (CBD: THC > 20:1) 100 mg/ml, available in 5 ml and 10 ml sizes. Initial dose: 1-3 mg/kg/day every 12 hours for 1 month, adjust CBD dosage once 1-5 mg every 1-2 weeks until seizures are controlled. And no side effects with a maximum dose of 20-25 mg/kg/day and the maximum adult dose is 600 mg per day, with THC < 0.5 mg/kg/day considered. (Reference to the guideline for the use of cannabis extracts in difficult-to-treat epilepsy of the Association of Pediatric Neurology (Thailand))

Do not use products containing THC [15-16]

1. Patients with a history of allergic reactions to cannabis extract products which may be caused by other components and/or substances are a solvent (solvent) used in extraction.

2. Severe cases of unstable cardio-pulmonary disease (angina, peripheral vascular disease, cerebrovascular disease, and arrhythmia) or risk factors for cardiovascular disease.

3. People with a history of psychosis or active mood disorder. Anxiety disorder.

4. Avoid using in pregnant women breastfeeding women of reproductive age who do not use contraception or women who planning to get pregnant because studies have shown that babies are born prematurely. Low birth weight infants, including cannabinoids in breast milk.

Drug Interactions of Active Cannabis Substances [15-16]

Other drugs that cause changes in blood levels of THC and CBD. Since THC and CBD are metabolized by several types of cytochrome P450 (cyp):

1. THC is metabolized by CYP2C9, CYP2C19, and CYP3A4.

2. CBD is metabolized predominantly by CYP2C19 and CYP3A4 and is metabolized a minority by CYP1A1, CYP1A2, CYP2C9 and CYP2D6.

Concomitant use of THC and CBD with other drugs that inhibit CYPP450, especially CYP2C19 and CYP3A4.For example, fluoxetine can increase THC and CBD blood levels, leading to side effects.

On the other hand, when THC and CBD are used together with enzyme inducers such as rifampicin, Carbamazepine lowers the levels of THC and CBD in the blood.

2. THC and CBD alter the levels of other drugs. Because THC and CBD have both enzyme inducer and enzyme inhibitor effects as follows:

2.1 THC has an induction effect on CYP1A2.

2.2 THC has an inhibitory effect on CYP2C9, CYP2D6 and CYP3A4, which may result in other drugs being metabolized. These CYPs produce higher levels of drugs such as warfarin (metabolized by CYP2C9).INR can be higher.

2.3 CBD has an inhibitory effect on CYP1A1, CYP1A2, CYP1B1, CYP2B6, CYP2C19, CYP3A4 and CYP2C9.Therefore, the concomitant use of CBD with other drugs metabolized by these CYPs, for example, warfarin, clobazam (metabolized by CYP3A4 and CYP2C19), fluoroquinolones (metabolized by CYP1A2), and dihydropyridines (metabolized by CYP3A4) to higher drug levels which may cause side effects

The concentration of active substances in marijuana can have a negative effect on health (Toxic dose). Usually using 1 drop of cannabis oil under the tongue. may receive different active substances in cannabis oil For example, bottle number 31 has a concentration of THC: CBD is 0:333.13mg/ml. In 1 drop, there is a concentration of CBD equal to 16.656mg/ml. bottle number 54 has a concentration of THC: CBD is 589.58: 89.24 mg/ml. In 1 drop, there is a concentration of THC: CBD equal to 29.79:4.462 mg/ml, and bottle number 32 has a concentration of THC: CBD is 0.00: 0.00 mg/ml. In 1 drop, there is a concentration of THC: CBD equal to 0 mg/ml

Therefore, medical marijuana users should be aware of drug interactions with other drugs that the patient is currently taking for patient safety.

Using cannabis oil without a clearly labeled ingredient cause health effects both aspects do not have good results in treatment, and may be poisoned by substances used in treatment due to non-standard production standards and no quality verification after production This can be seen from the concentration of cannabis compounds in commercial cannabis oil that ranges from the concentration of THC 0-1225.25mcg/ml and the concentration of CBD 0-524.47 mcg/ml

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Nil

Author's contributions

All the authors have contributed equally.

Conflicts of interests

The authors declared that no conflicts of interest.

Table 6 The concentration of delta-9-tetrahydrocannabinol: cannabinoid in commercial cannabis oil

No.	THC(mg/ml) Mean±SD	CBD(mg/ml) Mean±SD	No.	THC(mg/ml) Mean±SD	CBD(mg/ml) Mean±SD	No.	THC(mg/ml) Mean±SD	CBD(mg/ml) Mean±SD
1	128.17±3.28	8.36±5.41	21	0.00	225.25±2.58	41	20.02±9.88	1.25±8.64
2	325.02±1.18	46.98±12.36	22	420.12±1.32	240.24±5.22	42	0.00	0.00
3	132.13±6.84	6.55±1.25	23	406.06±0.58	0.00	43	30.54±1.39	22.58±1.88
4	492.36±6.86	120.58±5.65	24	426.32±12.73	83.46±7.10	44	156.58±5.33	85.54±4.69
5	596.87±12.56	205.69±2.99	25	464.14±3.41	9.07±7.16	45	200.85±4.36	56.69±8.11
6	119.16±2.01	5.87±8.66	26	58.62±2.01	0.00	46	0.55±0.70	0.00
7	643.12±14.68	240.87±5.66	27	27.00±3.63	0.00	47	45.88±6.33	5.84±2.22
8	40.01±0.56	4.58±9.33	28	151.93±7.84	20.60±9.06	48	612.45±5.55	89.88±4.87
9	20.89±6.38	1.48±6.58	29	148.01±2.01	19.62±9.70	49	325.52±6.33	45.96±9.55
10	129.15±0.58	12.56±1.85	30	138.88±8.04	21.32±4.25	50	0.12±2.10	0.00
11	426.32±6.55	132.37±10.56	31	0.00	333.13±1.19	51	64.54±8.58	12.24±7.69
12	122.54±2.68	7.89±5.65	32	0.00	0.00	52	20.22±5.69	0.00
13	46.41±1.11	2.68±0.12	33	1225.25±12.1	524.47±6.00	53	899.25±10.2	325.65±9.57
14	138.23±2.33	8.98±5.60	34	147.18±3.04	24.54±9.70	54	589.58±1.50	89.24±2.10
15	589.77±6.63	250.24±15.66	35	27.28±1.20	24.95±6.12	55	36.65±6.32	20.54±6.88
16	21.02±0.51	1.18±0.31	36	512.57±5.24	146.36±12.24	56	0.00	0.00
17	0.57±0.10	0.00	37	0.75±1.11	0.00	57	0.00	0.00
18	138.23±9.01	4.68±6.33	38	0.00	110.11±5.85	58	0.00	100.00±9.70
19	128.83±4.65	5.85±6.33	39	698.87±10.1	201.21±6.98	59	0.00	22.98±1.87
20	346.11±9.25	145.69±7.85	40	0.00	27.00±2.70	60	25.00±3.20	0.0

Abbreviation

Δ9-THC	Delta-9 tetrahydrocannabinol
CBD	Cannabidiol
CS	calibration standards
FDA	Food and Drug Administration
GPO	Government Pharmaceutical Organization
LOD	Limit of Detection
LOQ	Limit of Quantitation
PDA	ACQUITY UPLC® Photodiode Array (PDA) Detector (Waters Corporation, Milford, MA, USA)
QC	Quality Control
RSD	Relative Standard Deviation
THC-D3	delta-9 tetrahydrocannabinol-D3 (THC-D3) (internal standard, IS)
UPLC-PDA	Ultra performance liquid chromatography

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