การเปรียบเทียบตัวทำละลายที่ใช้ในการสกัดกัญชา สำหรับการวิเคราะห์ด้วยแก๊สโครมาโทรกราฟี-แมสสเปคโตรเมตรี

Comparison of extraction solvents for gas chromatography - mass spectrometric analysis of cannabis plants

สุมาลี ผาจันทร์[1], ประพิณ วิไลรัตน์[2] และ อจิรภาส์ พันธัย[1]*

Sumalee Phajan[1], Prapin Wilairat[2] and Achirapa Bandhaya[1]*

[1]*โครงการบัณฑิตศึกษานิติวิทยาศาสตร์ หลักสูตรนานาชาติ คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล*

[2]*ศูนย์ตรวจสอบสารต้องห้ามในนักกีฬา มหาวิทยาลัยมหิดล*

[1] *Forensic Science Graduate Programme, Faculty of Science, Mahidol University*

[2]*National Doping Control Centre, Mahidol University*

บทคัดย่อ

วัตถุประสงค์ของการศึกษาในครั้งนี้ คือ การประเมินประสิทธิภาพของตัวทำละลายที่ใช้ในการสกัดกัญชาที่วิเคราะห์ด้วยเทคนิคแก๊สโครมาโทรกราฟี-แมสสเปคโตรเมตรี โดยองค์ประกอบหลักของสารกลุ่มแคนนาบินอยด์ ได้แก่ delta-9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD) และ cannabinol (CBN) จะถูกแยกและ วิเคราะห์ปริมาณในตัวอย่างกัญชาแห้งที่สกัดด้วยเมทานอลหรือเฮกเซน เมื่อนำผลการวิเคราะห์เปรียบเทียบกัน พบว่า เมทานอลมีประสิทธิภาพดีกว่าเฮกเซน เนื่องจากเป็นตัวทำละลายที่มีความมีขั้วมากกว่า จากกราฟของสาร**ในกลุ่ม** แคนนาบินอยด์ พบว่า อยู่ในช่วง 20 - 200 ไมโครกรัม/มิลลิลิตร, สมการ y = 0.0214x - 0.2844 (R^2 = 0.997) สำหรับ Δ^9-THC, y = 0.0495x - 1.3427 (R^2 = 0.984) สำหรับ CBD และ y = 0.1037x - 1.0085 (R^2 = 0.999) สำหรับ CBN ค่าขีดจำกัดการตรวจวัดที่ได้ ของ Δ^9-THC, CBD และ CBN คือ 5.45, 12.1 และ 0.812 ไมโครกรัม/มิลลิลิตร ตามลำดับ

คำสำคัญ : Δ^9- tetrahydrocannabinol, Cannabidiol, Cannabinol, กัญชา

Abstract

The aim of this study was to evaluate the efficiency of solvent extraction of cannabis plants, with the analysis of the extracts carried out by gas chromatography-mass spectrometry (GC-MS). The three major cannabinoids, delta-9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD) and cannabinol (CBN) were separated, identified and determined quantitatively. Dried cannabis samples were extracted with either methanol or hexane and their results were compared. Methanol was found to be more efficient than hexane, since it is a more polar solvent. The calibration curves for the three cannabinoids were linear in the concentration range of 20 - 200 µg/mL, with equations y = 0.0214x - 0.2844 (R^2 = 0.997) for Δ^9-THC, y = 0.0495x - 1.3427 (R^2 = 0.984) for CBD and y = 0.1037x - 1.0085 (R^2 = 0.999) for CBN. The limits of detection (LOD) were 5.45, 12.1 and 0.812 µg/mL, respectively.

Keywords: Δ^9- tetrahydrocannabinol, Cannabidiol, Cannabinol, extraction solvent, hexane, methanol

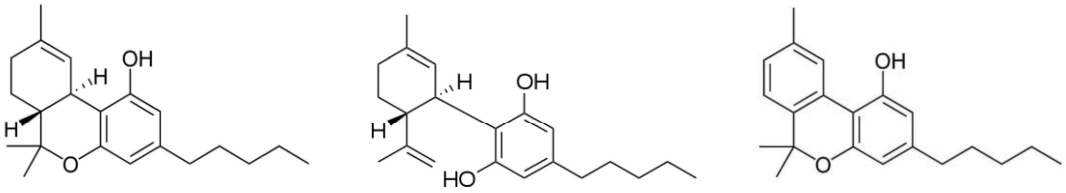
*Corresponding author. E-mail : achirapa.ban@mahidol.ac.th

1. Introduction

Cannabis or marijuana (*Cannabis sativa* L.) is one of the most well-known illicit drugs in the world. The plant is widely distributed but its cultivation is prohibited in most countries (Stefanidou *et al*., 1998), including Thailand. *Cannabis* has been a source of fiber, food, oil, medicine, and inebriant since prehistoric time as reported by Hillig and Mahlberg (2004). Plants in this genus produce a group of substances known as cannabinoids. The three main cannabinoids are psychoactive Δ^9- tetrahydrocannabinol (Δ^9-THC), and non-psychoactive cannabidiol (CBD) and cannabinol (CBN). *Cannabis sativa* L. is classified as a drug or fiber type based on morphology and chemical characteristics. Drug-type of C. sativa is commonly called “marijuana”, and is an addictive substance since it contains high THC content (> 0.3%). Fiber-type, or “hemp”, is cultivated for its fiber, and contains lower amount of THC content (< 0.3%) (Kojoma *et al*., 2006). According to the United Nationals Office on Drug and Crime (UNODC), the maximum permitted content of Δ^9-THC in cannabis is 0.3% dry weight (UNODC, 2009).

In Thailand, *Cannabis sativa* L. is classified as category V narcotic, according to section 7, Narcotics Act B.E. 2522 (1979), and the maximum permitted content of Δ^9-THC in cannabis is 0.3% dry weight.

In the extraction procedure, many types of organic solvents, such as hexane (Kallawicha *et al*., 2008), methanol (Tipparat *et al*., 2012) or a mixture of hexane and ethyl acetate (60:40, v/v) (Bruci *et al*., 2012) have been employed. In this research two solvents, methanol and hexane, were compared. The extracts of cannabis samples were analyzed by GC-MS, which provides high specificity and sensitivity (UNODC, 2013).



Delta-9-tetrahydrocannabinol (Δ^9-THC) | Cannabidiol (CBD) | Cannabinol (CBN)

Figure 1 Structures of Cannabinoid compounds in *Cannabis*

2. Objective of the study

To evaluate the efficiency of the solvent for extraction of cannabis plants using gas chromatography-mass spectrometry (GC-MS) analysis.

3. Materials and Methods

Sample preparation

Twenty milligrams (Kallawicha *et al*., 2008) of dried *Cannabis* samples were extracted with 1 mL of two different solvents, methanol or hexane for 5 minutes. Then, 500 µL of supernatant were transferred to a new vial and 10 µL of 1.0 mg/mL diphenylamine in methanol was added as the internal standard. The sample

was evaporated with N_2 stream and the dry residue reconstituted with 500 µL of ethyl acetate. All studies were performed in duplicate.

Standard solutions of Δ^9-THC, CBD, and CBN in methanol (Cerilliant, USA) were diluted to 0.2 mg/mL with methanol and 10 µL of 1.0 mg/mL diphenylamine (internal standard) in methanol were added.

Analysis of cannabinoids by gas chromatography-mass spectrometry

One microliter of each sample was injected to the GC-MS system. The analysis was performed using Agilent GC-MS 7890A/5975C (Agilent, USA) with Hewlett-Packard HP-5MS crosslinked 5% phenyl methyl silicone (30 m x 0.25 mm I.D., film thickness 0.25 µm) capillary column. The conditions of analysis was modified from Kallawicha *et al*. (2008) and were as follows: oven temperature: Initial temperature, 100ºC, ramping at 15ºC/minutes to 300ºC, holding for 8.00 minutes. Total run time 21.33 minutes; interface temperature 300ºC; split mode at ratio 20:1. The helium flow-rate was 1 mL/ minutes.

The results were analyzed by the ChemStation Integrator program (Agilent, USA). The peak area ratio between the cannabinoids and the internal standard was used for quantitation. In this study the characteristic ions for the cannabinoids were selected using published data in the literature. For Δ^9-THC the ions are m/z 299 (the quantifying ion), m/z 314 and m/z 231 (the qualifying ion). The ions for CBD are m/z 231 (the quantifying ion), m/z 174 and m/z 314 (the qualifying ion). For CBN the ions are m/z 295 (the quantifying ion), with m/z 238 and m/z 310 as qualifier ion. For diphenylamine, the internal standard, the ion is m/z 169 (the quantifying ion)(Kallawicha *et al*., 2008).

4. Results and Discussion

The results of the cannabinoid contents for the two extraction solvents are shown in Table 1. Samples THC01 and THC02 are dried cannabis samples that had been extracted with methanol. Samples THC03 and THC04 are dried cannabis samples extracted with hexane. The retention times of diphenylamine, CBD, Δ^9-THC and CBN were 5.942, 11.00, 11.48 and 11.81 minutes, respectively (see Figure 2).

The calibration curves for the three cannabinoids were linear over the concentration range of 20 - 200 µg/mL. The equations for the calibrations were $y = 0.0214x - 0.2844$ ($R^2 = 0.997$) for Δ^9-THC, $y = 0.0495x - 1.3427$ ($R^2 = 0.984$) for CBD and $y= 0.1037x - 1.0085$ ($R^2 = 0.999$) for CBN. The limit of detections (LOD) for Δ^9-THC, CBD and CBN were 5.45, 12.1 and 0.812 µg/mL, respectively.

This study showed the concentrations of Δ^9-THC, CBD, CBN in dried cannabis samples with different solvents extraction. The concentrations of Δ^9-THC, CBD, CBN in sample THC01 and THC02 were similarly. Samples THC03 and THC04 showed the concentration of Δ^9-THC, CBD, CBN in cannabis samples lower than sample THC01 and THC02. The results of the percentage of cannabinoid contents were showed similar to the concentrations. Therefore, from the results showed that extracted with methanol solvent was better than extracted with hexane.

Table 1 The concentrations of the extracted solution and calculated percentage cannabinoid content.

Samples ID	Concentration (µg/ml)			Cannabinoid content (% dry wt.)		
	Δ^9-THC	CBD	CBN	%Δ^9-THC	%CBD	%CBN
THC01	21.16 ± 0.84	43.51 ± 1.37	36.98 ± 19.85	0.104 ± 0.005	0.214 ± 0.005	0.182 ± 0.098
THC02	23.16 ± 0.58	49.26 ± 0.49	38.53 ± 27.17	0.115 ± 0.003	0.245 ± 0.003	0.192 ± 0.135
THC03	18.57 ± 1.05	39.78 ± 2.23	24.76 ± 14.61	0.092 ± 0.006	0.197 ± 0.113	0.123 ± 0.073
THC04	18.88 ± 0.05	40.28 ± 0.67	25.50 ± 15.84	0.093 ± 0.001	0.198 ± 0.001	0.126 ± 0.080

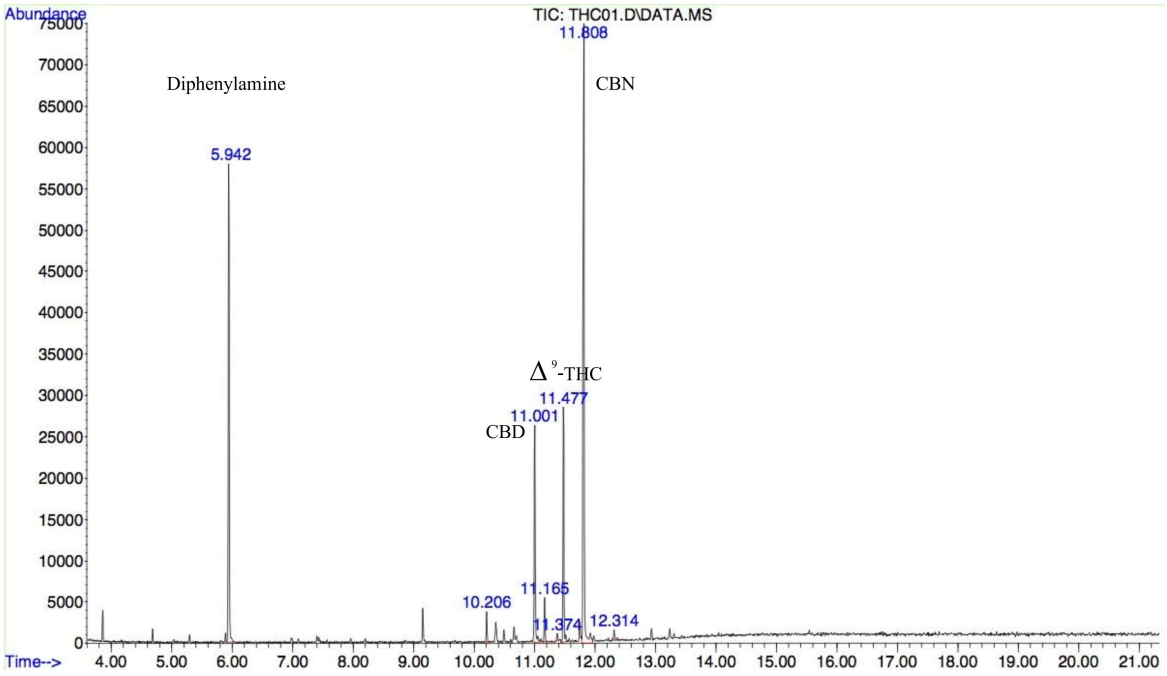


Figure 2 Total ion chromatogram of a standard solution of diphenylamine (internal standard), CBD, Δ^9-THC and CBN. The retention times were 5.942, 11.00, 11.48 and 11.81 minutes, respectively.

5. Conclusion

Using GC-MS technique, the concentrations of the cannabinoids in the extracting solution (1 mL solvent and 20 mg dry sample) were determined and the percentage contents of Δ^9-THC, CBD and CBN in the cannabis samples were calculated. The results showed that methanol is a more efficient solvent than hexane due to its higher polarity.

6. Acknowledgements

This study was supported by the Armed Forces Research Institute of Medical Sciences (AFRIMS).

7. References

Bruci, Z., Papoutsis, I., Athanaselis, S., Nikolaou, P., Pazari, E., Spiliopoulou, C. and Vyshka, G. (2012). First systematic evaluation of the potency of *Cannabis sativa* plants grown in Albania. *Forensic Science International*, 222, 40-46.

Hillig, KW. and Mahlberg, PG. (2004). A chemotaxonomic analysis of cannabinoid variation in Cannabis (Cannabaceae). *American Journal of Botany*, 91(6), 966-75.

Kallawicha, K., Bunyapraphatsara, N., Wilairat, P. and Panvisavas, N. (2008). Survey of Cannabis sativa L. "Fiber Type" Cultivated in Thailand Using Gas Chromatography-Mass Spectrometry. *Srinakarinwirot science journal*, 24(1), 153-64.

Kojoma, M., Seki, H., Yoshida, S. and Muranaka, T. (2006). DNA polymorphisms in the tetrahydrocannabinolic acid (THCA) synthase gene in "drug-type" and "fiber-type" Cannabis sativa L. *Forensic Science* International, 159(2-3), 132-40.

Stefanidou, M., Dona, A., Athanaselis, S., Papoutsis, I. and Koutselinis, A. (1998). The cannabinoid content of marihuana samples seized in Greece and its forensic application. *Forensic Science International*, 95, 153-62.

The Narcotics Act of B.E. 1979. Food and Drug Administration, Thailand

Tipparat, P., Natakankitkul, S., Chamnivikaipong, P. and Chutiwat, S. (2012). Characteristics of cannabinoids composition of Cannabis plants grown in Northern Thailand and its forensic application. *Forensic Science International*, 215(1-3), 164-70.

UNODC. (2009). Recommended methods for the identification and analysis of cannabis and cannabis products. Vienna: United Nations publication.

UNODC. (2013). Recommended methods for the Identification and Analysis of Synthetic Cannabinoid Receptor Agonists in Seized Materials. Vienna: United Nations publication.