American Journal of Biomedical and Life Sciences
2016; 4(1): 1-10
Published online March 9, 2016 (http://www.sciencepublishinggroup.com/j/ajbls)
doi: 10.11648/j.ajbls.20160401.11
ISSN: 2330-8818 (Print); ISSN: 2330-880X (Online)

Analysis of User's Hair Cannabinoid of Narcotic Type of Marijuana (*Cannabis Sativa L.*) Using GCMS Technic

Muhammad Taufik[1], Harlem Marpaung[2], Jamaran Kaban[2], Basuki Wirjosentono[2]

[1]Medan Region Police Department, North Sumatra, Medan, Indonesia
[2]Faculty of Mathematics and Science, University of North Sumatera, Medan, Indonesia

Email address:
tofikmuhammadusu@gmail.com (M. Taufik)

To cite this article:
Muhammad Taufik, Harlem Marpaung, Jamaran Kaban, Basuki Wirjosentono. Analysis of User's Hair Cannabinoid of Narcotic Type of Marijuana (*Cannabis Sativa L.*) Using GCMS Technic. *American Journal of Biomedical and Life Sciences.* Vol. 4, No. 1, 2016, pp. 1-10.
doi: 10.11648/j.ajbls.20160401.11

Abstract: This paper describes the analysis of cannabinoids in the hair of the user of the narcotic type of marijuana *(Cannabis sativa L)*, using the technique Gas Chromatography Mass Spectroscopy (GCMS) and the validation of the method used. This research was conducted through experiments. Samples were taken from users of narcotic type of marijuana, found in Al-Kamal Sibolangit Rehabilitation Centre, North Sumatra, Indonesia. Marijuana plants were taken from the evidence in Forensic Laboratory of Medan Branch Police. Extraction is done through maceration by sonication at 42 KHz and using methanol – 2 propanol (1:1), kloroform, and petroleum benzene each of 2 ml for 5 minutes. Testing is done with fast blue salt B test and show there is a precipitate Violet. Optimal results obtained at a temperature of 50°C extraction. Identification is done with Thin Layer Chromatography (TLC) in the mobile phase of toluene and n-hexane (1:1) under alkaline conditions of pH 9,5, followed by GC MS techniques for sixteen (16) minutes. The results are contained compounds of cannabidiol, cannabinol, and Δ^9THC (Tetrahydrocannabinoid) with concentrations ranging from 0.25 up to 2.82 ng/mg in the hair of the user. Validation of GC MS method for the compounds of cannabidiol, cannabinol, and Δ^9THC produces an accuracy value with *%recovery* is 103,83, 102,67, and 101,17 respectively. Precision test produces a value of Relatif Standard Deviation (RSD) = 1,3058%, 0,8997%, dan 0,8997% respectively. Linearity test generate r value each of the regression is 0,922, 0,955, and 0,921. Limits of Detection (LOD) is obtained 0,000168 ng/mg, 0,000117 ng/mg, and 0,000164 respectively. Limits of Quantification (LOQ) is 0,00056 ng/mg, 0,00039 ng/mg, and 0,00054 ng/mg respectively. This indicates that the modification process of extraction and GC MS techniques used could produce cannabinoid compounds (cannabidiol, cannabinol, and Δ^9THC) in hair samples quickly and accurately.

Keywords: Analiysis, Hair, Marijuana *(Cannabis sativa L)*, GCMS, KLT, Validation

1. Introduction

Narcotics is a drug that in small doses dulls the senses, relieves pain, and brings on sleep but in larger doses has dangerous effects, that includes some (as morphine) that are used in medicine and others (as heroin) that are used illegally, often and that causes addiction. Some of these compounds have been shown to have complex biological effects on various systems, including the reproductive system. It also effect gonadal function [1, 2]. Marijuana *(Cannabis sativa L)* is a class of drugs that some countries prohibit the use of this material freely. There are certain areas in some countries that use as an ingredient in cooking [3]. Another use is also often together with heroin, sometimes mixed with tea to drink [4].

Indonesia banned the use of narcotics is not legal, its regulated in No. 35 of Year 2009 of narcotics laws. But the circulation of narcotics is still ongoing due to several reasons including profits earned by the seller and the enjoyment felt by its users. National Narcotics Agency of Indonesia released that the number of drug users has been very worrying, reaching some 5.8 million peoples, with 4.2 million peoples who are users of marijuana in Indonesia. In North Sumatra, there are some 288.226 peoples who become drug users, including as many as 192.590 peoples are cannabis users. There are as many as 104.269 of students who use drugs, and there are as many as 87.800 people are users of cannabis [5]. The prohibition against the use of this material freely in Indonesia is due to its impacts, ie dependence on its use (Lestari, 2013) [6]. Marijuana plants form looks like Figure 1a and 1b.

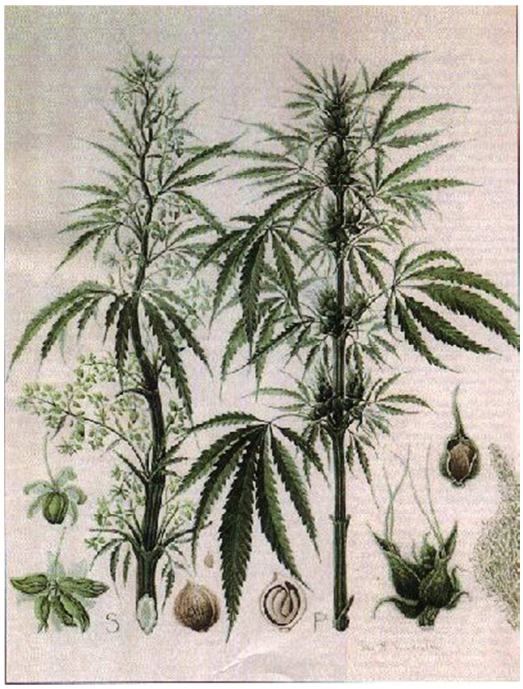

Figure 1a. *Marijuana plants.*

Figure 1b. *Dried marijuana.*

This plant is included cannabaceae family, namely the species *Cannabis sativa L*. Active chemical in marijuana is Δ^9tetrahydrocanabinol (THC), Cannabidiol, cannabinol, Cannabicyclol, Cannabichromene, Cannabivarine, and another cannabinoid. Generally there are four types of cannabinoid, ie Tetrtahydrocannabinol (THC), Cannabidiol (CBD), Cannabichromene (CBC), and Cannabigerol (CBG) [34]. The chemical structure and biosynthetic pathway of marijuana is drawn as Figure 2 (a) and (b).

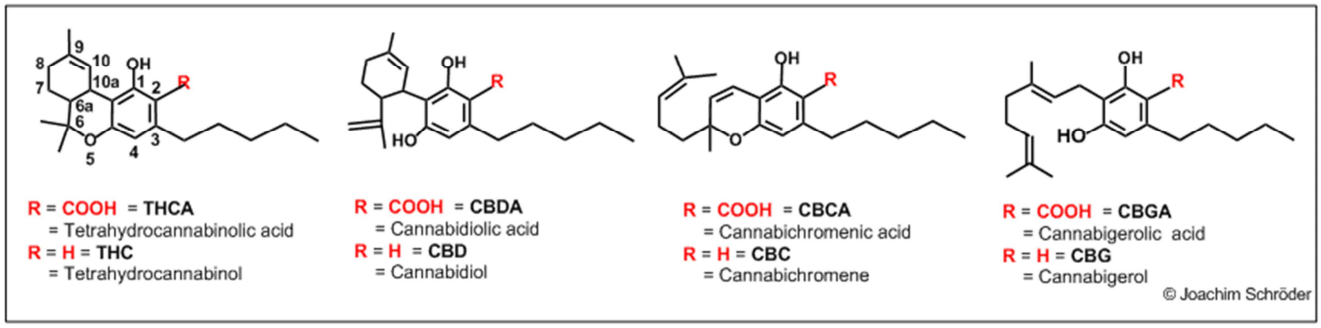

Figure 2a. *The chemical structure of the type of cannabinoid in Cannabis sativa L.*

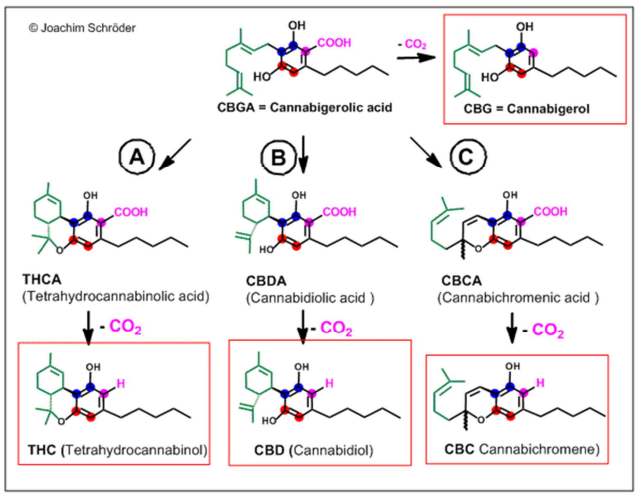

Figure 2b. *Biosynthetic pathway THC, CBG, CBD dan CBC.*

Cannabis sativa L is one of the species of cannabis plant, which contains a compound of cannabinoid, which may activate cannabinoid receptors to the wearer to experience a sense of euphoria (Reggra, 2009; [7, 8]. When THC is bound to proteins, it will stimulate the reaction of nerve cells, causing the patient wishes to use it continuously [9]. Short term effect of marijuana is considering learning disorders, disorders of perception, difficulty thinking and solving problems, uncoordinated movement, increased heart rate and panic [10]. Working THC is disturbing memory system and can cause nervous incoordination and loss of balance [11, 12] and can cause impaired cardiovascular [13].

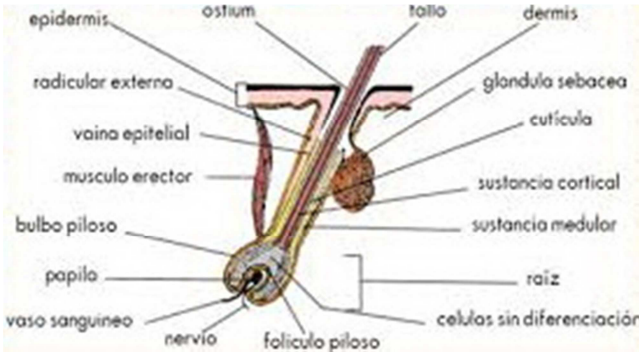

Figure 3. *Anatomy of Hair.*

When this material is consumed, then the Cannabinoid compounds contained in marijuana, binds to cysteine of hair after seven days of use by the user. With increasing time will continue up to the end of the hair. It can provide information on how long a person to consume narcotics, and will be readily history of the use of narcotics. Anatomy of hair looks like figure 3. The protein contained in human hair is a kind of cysteine, the amino acid compounds that have an element of sulfide in an amount high enough percentage, ie 90%. -S-S- disulfide bridges of cysteine is one of the major factors in the formation of hair [14]. Hair growth in adult humans is influenced by sex hormones which has two phases, namely the anagen growth and katagen. This is a type of hormone androgen, estrogen, thyroxine, and corticosteroids [3, 15].

In laboratory tests required methods which is tested with optimal results [16] because the test sample is very complex, covering the marijuana plant itself, the compounds synthesized and isolation, foodstuffs and beverages containing marijuana, and also compounds from the metabolites of users [17]. Inspection methods narcotic type of marijuana is still very limited, given in several States legalized marijuana use. Things are different in Indonesia, users and dealers of marijuana illegally will be subject to criminal sanctions. Therefore, the required standard method in the analysis of narcotic type of marijuana to ascertain whether a person is never used drugs or not.

Several techniques have been developed, in analyzing narcotics from the hair of which is a chromatographic technique using Gas Chromatography Mass Spectroscopy (GCMS), Liquid Chromatography Mass Spectroscopy (LCMS) and the Radio Immuno Assay (RIA) test. Such techniques through a compound of arsenic in the hair, determining amphetamines in hair hamster through RIA. The use of RIA technique has its limitations because it is less sensitive [18]. GCMS technique has the advantage particularly for natural compound volatile material. Mechanical High Performance Liquid Chromatography (HPLC) and LCMS require a long time in preparation and analysis when compared with GCMS [14].

The solvent used is methanol, n-hexane: ethyl acetate (9: 1) and cyclo hexane. THC obtained is 0.3 up to 2.3 ng/mg. In this case, preparation and extraction still takes 2 days and with GCMS technique is for 30 minutes and cannabinoid compound that is detected is just THC. [19, 20, 21] have also been analyzed cannabinoid hair samples. The solvent used is ethyl acetate: isopropanol (8.5: 1.5 v / v), methanol, and petroleum ether. Compounds that are analyzed are limited to THC at a concentration of 2 ng/mg. [15] using dichloromethane solvent and require preparation time for 3 days and GCMS technique for 60 minutes. [22] using methanol and 1 day preparation time and with GCMS technique is 40 minutes. Laboratory examination methods still rely on simple spot test with results that are less accurate [23]. For these reasons, the analysis of hair of drug users of cannabinoid type of marijuana *(Cannabis sativa L)* is developed by maceration and sonication, and GCMS technique is used for determining the content of cannabinoids from cannabis in the hair.

2. Method

2.1. Preparation and Extraction

This study used an experimental method for extracting cannabinoids from hair samples by maceration and sonication, and without sonication at various temperatures of 30°C, 40°C, 50°C, and 60°C. Levels of optimum results is determined by a descriptive method using GCMS technique. The sample of drug users hair is extracted by maceration, and samples of the marijuana plant *(Cannabis sativa L)* is taken as the benchmark. Preliminary test use Reagens fast blue salt B test and Thin Layer Chromatography (TLC), that followed by assay techniques cannnabinoid with GCMS [24] and then validating the methods of instrumentation.

Cannabis sativa L as the comparison is taken as much as 50 grams of some of the evidence gathered by the Police Forensic Laboratory Branch of Indonesia in Medan, cleaned, and dried naturally. This material is dried in the open air for 24 hours, then weighed as much as 50 mg. It is extracted by maceration using methanol - 2-propanol (1: 1) for 5 minutes, did sonication using ultrasonic bath at 42 KHz, and then filtered and dried. The extract was diluted with methanol to a volume of 10 ml, and the pH was made 9.5 [25]. Qualitative test is done by Reagens fast blue salt B test and Thin Layer Chromatography. A total of 1 mL sample was taken and injected into GCMS to confirm cannabinoid compounds contained in the *Cannabis sativa L*.

Hair samples were taken from the hair of people who cared for in Al - Kamal Rehabilitation Centre in Sibolangit, ie the hair 14 days, 30 days, and 60 days after using of narcotics. Stages of the collection is done in four phasem namely phase 1, the collection of hair samples of 40 mg (± 40 pieces), which are taken from the head in the front of the user. Phase 2, hair cut with spacing along the 2.5 cm from the bottom, followed by storage. Phase 3, the storage in aluminum foil. Phase 4, labeling to prevent the exchanged sample, by giving the code number.

Preparation was conducted in the Graduate's Laboratory of Chemistry of University of North Sumatra, and GC MS analysis was conducted in the Laboratory Research PT. Berca Commerce Medika Jakarta. The chemicals used are chemicals with a degree of purity of pro analysis. For the purposes of chromatography is used ultra pure chemicals with specification pro chromatography, which is 2-propanol, chloroform, methanol, petroleum benzene, toluene, n-hexane, and reagens fast blue test salt b. A total of 40 mg of hair is weighed and washed with methanol, and then dried by open air. Samples were cut into small pieces, with the size of 1-2 mm. Extraction was done by maceration (soaking) in a beaker with sonication process in the ultrasonic bath (frequency 42 KHz). The first extraction is performed for 5 minutes with 2 ml of a mixture of methanol - 2 propanol (1: 1), then extraction and sonication was continued for 5 minutes with klofororm, then extraction and sonication was

resumed for 5 minutes with petroleum benzene. The total time required is 15 minutes. In this extraction process to be varied by sonication and without sonication. In the extraction process by sonication temperature varied at 30°C, 40°C, 50°C, and 60°C. Then the solvent is allowed to evaporate at room temperature. Once evaporated, methanol is added to achieve a volume of 10 ml and filtered. The results of extraction are identified by the qualitative test using Reagens fast blue salt test B, followed by Thin Layer Chromatography (TLC), and as many as 1 mL sample was taken, and injected into GCMS for the determination of levels and then performed data interpretation. The same way is done on the hair which is extracted without sonication (Moosman and Auwarter, 2013).

2.2. Qualitative Test of Extraction

Qualitative test against the extraction of marijuana plant *(Cannabis sativa L)* and the hair, is then tested using Reagens fast blue salt b test and Thin Layer Chromatography (TLC) with 2 drops of sample is placed on a test plate reagens fast blue test salt. Violet precipitate showed a cannabinoid, which is divided into +, ++, and +++.

2.3. Thin Layer Chromatography (TLC) Test

Testing is done using Chromatography Chamber, namely with liquid toluene mobile phase n-hexane (1: 1). Plate TLC silica gel GF 254 with a size of 10 cm x 20 cm was heated in an oven at a temperature of 80°C for 15 minutes. The test sample is spotted to a TLC plate using a capillary tube. Reagens that fast blue stain test salt b showed is used to produce a violet color on a TLC plate. Value Retardation factor (Rf) is calculated using the formula:

$$\text{Rf} = \frac{\text{Distance of Substance}}{\text{Distance of Solvent front}} \quad (1)$$

2.4. GCMS Analysis

Cannabinoid standard solution of 34014 1000 ug/mg contains Cannabidiol, Cannabinol, and Δ9Tetrahydrocannabinol, is made in a concentration of 0.5, 1, 1.5, 2, and 2.5 ng/mg. The solvents used are methanol p.a. mercks artificial. At pH 9.5, each standard solution is injected into GCMS Agylent 7890 [26].

The cannabis plant samples is used to confirm the cannabinoid compounds contained in marijuana plant. The sample used as much as 1 mL and injected into GCMS instrument. 1 mL of extracted of marijuana user hair were assayed using GCMS technique. Specifications tool used is Gas Chromatography (GC) Agilent which combined with Mass Spectroscopy (MS) 7890 model. The column used is HP 5ms inside diameter (ID) of 0.25 mm and a film thickness of 0.25 mL [15]. Helium carrier gas at a constant rate of 1.5 ml/min [20]. Model splitles with a time of 15 seconds. Injector temperature is 250°C, and 265°C of interface temperature. Oven temperature started on 40°C and hold for 10 minutes at a temperature of 140°C, and increased to 280°C. The total time required is 16 minutes.

Validation of methods is done with test accuracy, linearity, limit of detection and the limit of quantification. Recovery test is conducted by the method of addition. This method is done by adding a number of standard solutions of Cannabidiol, Cannabinol, and Δ9 THC concentration of 1 ng/mg of hair samples of drug users at any time after 14 days of usage, that is Cannabidiol = 0.87 ng/mg, Cannabinol = 0.66 ng/mg, and Δ9 THC = 0.62 ng/mg, which made 6 (six) times replication. As much as 1.0 mL injected into GCMS [27]. The percentage of recovery is determined by determining what percentage of analyte is added earlier can be recovered by calculating the formula:

$$\%\,\text{Recovery} = \frac{(CF - CA)}{C * A} x100\,\% \quad (2)$$

Description:

CF = concentration of analyte obtained from measurements after the addition of the raw material (standard)

CA = concentration of the analytes prior to the addition of the raw material (standard)

C * A = Concentration of raw material (standard) is added [28].

Precision test is performed through the test recovery. Cannabinoid standard solution made of 0.5 ng/mg for 6 times. Then the response was measured using GCMS. Relative Standard Deviation (SDR) is calculated with the formula:

$$SD = \frac{SD}{\bar{x}} x\ 100\% \quad (3)$$

Description:

$\bar{x}$ = Sample average levels

SD = Standard Deviasi

RSD = Relatif standard deviation

Concentration of Cannabinoid standard solutions is determined in 0.5 mg/mg, 1 ng/mg, 1.5 ng/mg, 2 ng/mg, and 2.5 ng/mg. It is injected into GCMS instrument in accordance with operational procedures. Furthermore, the straight line equation is determined and set r value [29]. Linearity is determinated using the formula:

$$Y = bx + a \quad (4)$$

x = consentration

b = slope

a = intersept

Y = MS absorption

Limit of detection (LOD) and Limit of quantification(LOQ) is determined based on the Deviation Standard value, yaitu

$$LOD = \frac{3SD}{b};\ LOQ = \frac{10SD}{b}. \quad (5)$$

Description:

SD = Deviation Standard

b = Slope

3. Results and Discussion

Cannabis is extracted through maceration and sonication at 42 KHz with methanol - 2-propanol (1: 1) for 5 minutes. Results of Reagens salt b fast blue test showed a cannabinoid compound produce a purple precipitate, as presented in Table 1.

Table 1. *Preliminary test results of marijuana plant (Cannabis sativa L.).*

No	Sample	*Fast blue test salt b result*	Description
1.	Marijuana	Violet	Cannabinoid (+++)

Fast blue salt B test is used to determine reagensia cannabinoids in the marijuana plant *(Cannabis sativa L),* because it has the ability to oxidize cannabinoid compounds in marijuana plants and produces sharp color violet [30, 31, 32]. Confirmation test is performed at 1 mL sample of marijuana *(Cannabis sativa L)* to ensure cannabinoid compounds. GC chromatogram test results appear as shown in Figure 4.

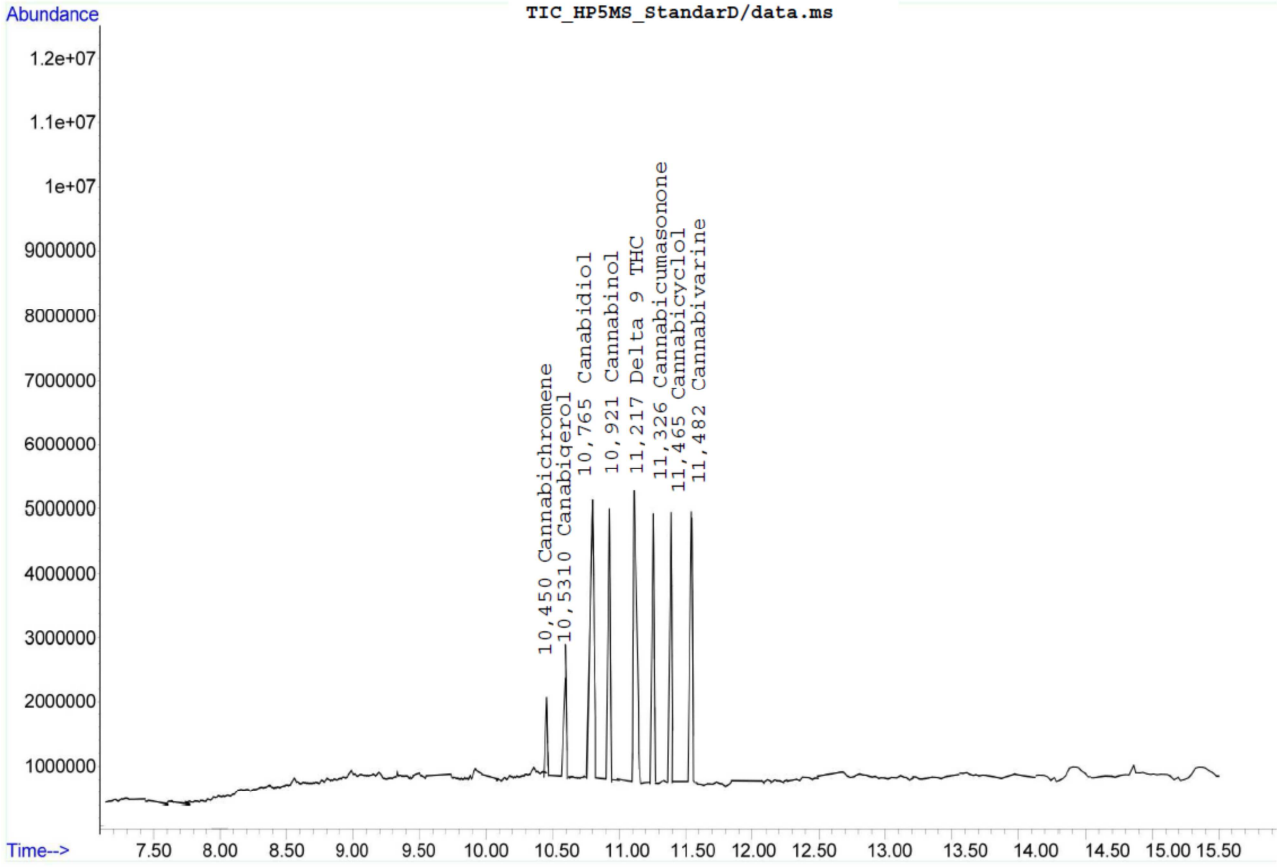

Figure 4. *Chromatogram cannabinoids in the marijuana plant (cannabis sativa L.).*

The cannabinoid extracted from the leaves of the cannabis plant *(Cannabis sativa L)* is one terpenophenolic group of compounds and secondary metabolites, which have special effects to humans. This is in line with the findings of Grotenhermen [33]. The resulting cannabinoid compound is *cannabichromene, cannabigerol, cannabidiol, Cannabinol, Δ^9 THC, cannabicumaronone, cannabicyclol, dan cannabivarine*. It is also in accordance with the results of the study reported by [34], that leaves marijuana *(Cannabis sativa L)* containing cannabinoid compounds that belonged terpenoids. MS spectrum of 8 (eight) compound indicates that there are Cannabicromene, Cannabigerol, Cannabidiol, Cannabinol, Δ^9 THC, Cannabicumaronone, *Cannabicyclol,* Cannabivarine on marijuana leaves. This is in line with the findings of [9, 34]. Compounds contained in hair samples, known through observations in a sustainable manner to aspects that affect the entry of the active compound. In extracting narcotic substances in hair by maceration is optimized by Ultrasonic Bath through the process of sonication at a frequency of 42 KHz to pull cannabinoid compounds found in the hair matrix [35]. Hair samples were extracted by sonication with methanol - 2propanol (1: 1) for five (5) minutes, followed by chloroform extraction for five (5) minutes, and lastly with petroleum benzene for 5 (five) minutes. The temperature variation performed at 30°C, 40°C, 50°C, and 60°C. The indicators of optimal extraction process are qualitatively showed there are deposits of violet after the addition of fast blue reagensia salt b test. The results obtained are presented in Table 2.

The optimal value of the extraction process is used in the sonication at a temperature of 50°C. This is consistent with the results obtained by [36], namely the terpenoid compounds with the highest randemen obtained at 50°C which is about 89%. Temperature 50°C is the optimum

temperature for the enzyme cysteine contained in the hair, so it helps the process of extracting cannabinoids in the hair [37]. The use of ultrasonic waves with a frequency of 42 kHz is very helpful in speeding up the contact time between sample and solvent. By the use of sonication, then the process of mass transfer of the compound to the solvent is to be faster. Sonication rely on wave energy that causes the formation of small bubbles due to the transmission of ultrasonic waves to help the diffusion of the solvent into the cell wall.

The influence of sonication may reduce clotting. This proves that the wave at 42 KHz at sonication methods can separate particle agglomeration and so there are many voids between the particle separator [38]. Extraction without using sonication is not showing real violet precipitate. This shows that the method of sonication have a very important role in extracting cannabinoids in hair samples. Thin layer chromatography (TLC) was used to detect the presence of cannabinoids in hair samples [37]. Rf value is calculated based on the distance stain (color violet) with a distance of solvent. In this study, the solvent used is toluene and n-hexane at alkaline pH of 9.5 [39].

Table 2. *The extraction optimization test results of hair samples.*

Day	Consumer	*Fast blue test salt b result*							
		Sonication				**Without Sonication**			
		30°C	**40°C**	**50°C**	**60°C**	**30°C**	**40°C**	**50°C**	**60°C**
14	1	+	+	++	+	-	-	-	-
	2	+	+	++	+	-	-	-	-
	3	+	+	++	+	-	-	-	-
	4	+	+	++	+	-	-	-	-
	5	+	+	++	+	-	-	-	-
	6	+	+	++	+	-	-	-	-
	7	+	+	+	+	-	-	-	-
	8	+	+	++	+	-	-	-	-
	9	+	+	++	+	-	-	-	-
	10	+	+	++	+	-	-	-	-
30	1	+	+	++	+	-	-	-	-
	2	+	+	++	+	-	-	-	-
	3	+	+	+	+	-	-	-	-
	4	+	+	++	+	-	-	-	-
	5	+	+	++	+	-	-	-	-
	6	+	+	++	+	-	-	-	-
	7	+	+	+	+	-	-	-	-
	8	+	+	++	+	-	-	-	-
	9	+	+	+	+	-	-	-	-
	10	+	+	++	+	-	-	-	-
60	1	+	+	++	+	-	-	-	-
	2	+	+	++	+	-	-	-	-
	3	+	+	++	+	-	-	-	-
	4	+	+	++	+	-	-	-	-
	5	+	+	+	+	-	-	-	-
	6	+	+	++	+	-	-	-	-
	7	+	+	++	+	-	-	-	-
	8	+	+	++	+	-	-	-	-
	9	+	+	+	+	-	-	-	-
	10	+	+	++	+	-	-	-	-

+ = cannabinoid

Rf value of hair samples is obtained in the range of 0.425 to 0.450. This value is not much different from the value of 0.450. Reagens as the show of the fast blue stain test B was developed to clarify the appearance of violet color on a TLC plate. Rf value in Thin-Layer Chromatography is used as a parameter qualitative analysis to detect cannabinoid compounds [32, 33, 40].

GC MS technique was developed to detect the type of cannabinoid, which is found in hair samples. Conditions of GCMS instrument is used and observed through direct observation to obtain optimal results. In this study, the instrumentation used is Gas Chromatography (GC) coupled with Agilent Mass Spectroscopy (MS) 7890 models, HP 5ms column with a 0.25 mm OD, and 0.25 mL of film thickness [15]. Helium carrier gas at a constant rate of 1.5 ml/min. Model splittles with a time of 15 seconds. Injector temperature is 250°C and 265°C of interface temperature. Oven temperatures ranging from 40°C then detained at 140°C for 10 minutes and increased to 280°C at a rate of 10°C /min for 15 minutes [41].

Standard solution used is cannabidiol, cannabinol, Δ^9 THC with varying concentrations of 0.5 ng/mg, 1 ng/mg, 1.5 ng/mg, 2 ng/mg, and 2.5 ng/mg [30]. Volume of solution injected to GCMS instrument is as much as 1 mL. GCMS quantification based on the data, it appears that the levels of cannabinoids contained in the hair is a narcotic type of marijuana *(Cannabis sativa L)* is shown in Table 4.

Table 3. *Rf value on Thin Layer Chromatography (TLC).*

Day	Consumer	*Retardation Factor (Rf) value*															
		Sonication								Without Sonication							
		30°C		40°C		50°C		60°C		30°C		40°C		50°C		60°C	
		S	P	S	P	S	P	S	P	S	P	S	P	S	P	S	P
14	1	0,427	0,425	0,438	0,428	0,426	0,431	0,425	0,425	-	-	-	-	-	-	-	-
	2	0,428	0,425	0,426	0,428	0,428	0,431	0,428	0,425	-	-	-	-	-	-	-	-
	3	0,428	0,425	0,428	0,428	0,428	0,431	0,428	0,425	-	-	-	-	-	-	-	-
	4	0,427	0,425	0,428	0,428	0,426	0,431	0,426	0,425	-	-	-	-	-	-	-	-
	5	0,428	0,425	0,438	0,428	0,429	0,431	0,428	0,425	-	-	-	-	-	-	-	-
	6	0,427	0,425	0,419	0,428	0,428	0,431	0,428	0,425	-	-	-	-	-	-	-	-
	7	0,428	0,425	0,426	0,428	0,428	0,431	0,426	0,425	-	-	-	-	-	-	-	-
	8	0,428	0,425	0,429	0,428	0,428	0,431	0,426	0,425	-	-	-	-	-	-	-	-
	9	0,425	0,425	0,425	0,428	0,425	0,431	0,425	0,425	-	-	-	-	-	-	-	-
	10	0,431	0,425	0,425	0,428	0,428	0,431	0,425	0,425	-	-	-	-	-	-	-	-
30	1	0,440	0,425	0,438	0,428	0,426	0,431	0,425	0,425	-	-	-	-	-	-	-	-
	2	0,450	0,425	0,425	0,428	0,426	0,431	0,428	0,425	-	-	-	-	-	-	-	-
	3	0,431	0,425	0,426	0,428	0,428	0,431	0,428	0,425	-	-	-	-	-	-	-	-
	4	0,440	0,425	0,428	0,428	0,426	0,431	0,426	0,425	-	-	-	-	-	-	-	-
	5	0,425	0,425	0,425	0,428	0,429	0,431	0,428	0,425	-	-	-	-	-	-	-	-
	6	0,444	0,425	0,413	0,428	0,428	0,431	0,428	0,425	-	-	-	-	-	-	-	-
	7	0,440	0,425	0,425	0,428	0,426	0,431	0,426	0,425	-	-	-	-	-	-	-	-
	8	0,428	0,425	0,428	0,428	0,428	0,431	0,426	0,425	-		-	-	-	-	-	-
	9	0,425	0,425	0,425	0,428	0,428	0,431	0,425	0,425	-		-	-	-	-	-	-
	10	0,419	0,425	0,425	0,428	0,426	0,431	0,425	0,425	-		-	-	-	-	-	-
60	1	0,419	0,425	0,425	0,428	0,425	0,431	0,425	0,425	-	-	-	-	-	-	-	-
	2	0,431	0,425	0,425	0,428	0,426	0,431	0,428	0,425	-	-	-	-	-	-	-	-
	3	0,431	0,425	0,425	0,428	0,426	0,431	0,428	0,425	-	-	-	-	-	-	-	-
	4	0,419	0,425	0,419	0,428	0,426	0,431	0,425	0,425	-	-	-	-	-	-	-	-
	5	0,425	0,425	0,425	0,428	0,428	0,431	0,428	0,425	-	-	-	-	-	-	-	-
	6	0,425	0,425	0,413	0,428	0,426	0,431	0,428	0,425	-	-	-	-	-	-	-	-
	7	0,437	0,425	0,419	0,428	0,426	0,431	0,426	0,425	-	-	-	-	-	-	-	-
	8	0,419	0,425	0,413	0,428	0,428	0,431	0,425	0,425	-	-	-	-	-	-	-	-
	9	0,431	0,425	0,425	0,428	0,426	0,431	0,425	0,425	-	-	-	-	-	-	-	-
	10	0,431	0,425	0,425	0,428	0,425	0,431	0,425	0,425	-	-	-	-	-	-	-	-

Table 4. *Cannabinoid levels in the hair of the user.*

Consumer	Cannabidiol levels (ng/mg)			Cannabinol level (ng/mg)			Δ^9 THC (ng/mg) level		
	Day14	Day30	Day60	Day14	Day30	Day60	Day14	Day30	Day60
1	0,87	0,84	0,80	0,66	0,66	0,65	0,62	0,61	0,60
2	0,81	0,80	0,71	0,80	0,49	0,45	2,82	1,43	1,40
3	0,91	0,90	0,75	1,46	1,43	1,40	0,19	0,19	0,18
4	1,11	1,00	0,93	1,11	1,11	1,10	0,82	0,82	0,81
5	0,76	0,70	0,60	1,31	1,25	1,20	1,21	1,20	1,10
6	0,76	0,76	0,75	1,01	1,00	0,95	1,30	1,28	1,25
7	0,80	0,78	0,70	0,28	0,27	0,25	0,89	1,87	0,86
8	0,94	0,93	0,87	0,30	0,28	0,27	0,68	0,66	0,65
9	0,89	0,84	0,81	0,28	0,28	0,27	0,14	0,14	0,12
10	0,81	0,75	0,71	0,66	0,65	0,65	1,21	1,20	1,15

Levels of the compound cannabidiol decreased starting from day 14 to day 60 after use. Likewise with cannabinol compounds and Δ^9 THC. This is caused by the drug users were given the herbs that can lower the levels of cannabinoids during the first two months continuously. These data indicate that the hair can be used as a calendar in the history of drug use. Likewise that the time required for the extraction was for 15 minutes and timing analysis using GCMS instrument for 16 minutes. It is shorter when compared with previous studies by [15, 19, 20, 21].

Cannabinoid compounds are analyzed in this study, is based on the standard sample provided, namely cannabidiol (td = 150°C), cannabinol (bp = 180°C), and Δ^9 THC (td = 220°C). The three cannabinoid compounds are one group in compounds terpenoids, which is contained in the plant *Cannabis sativa L*. These plants if consumed by humans will stimulate the central nervous system and after the process of metabolism by the body, will be contained in metabolites such as urine, blood, hair, nails, saliva, and other secondary metabolites [35, 42]. Cannabidiol compounds was detected at m/e of 314, 299, 271, 231, 193, 135, and 91. This is consistent with MS spectra contained in the standard solution.

Addition method is a way to identify% recovery, in particular by adding a number of Cannabidiol standard solution, cannabinol, and Δ^9 THC, with a concentration of 1 ng/mg in samples of drug users hair extraction of patient after 14 days of use, ie. Cannabidiol = 0.87 ng/mg, cannabinol = 0.66 ng/mg, and Δ^9 THC = 0.62 ng/mg), which is conducted for 6 (six) times replication and 1.0 mL injected into GCMS. The results of the determination of the accuracy of cannabidiol, cannabinol, and Δ^9 THC shown in Table 5, Table 6 and Table 7. Each of them had an average of 103.83; 102.67; and 101.17. The value meets the acceptance criteria of% recovery is 70% up to 120% [28, 43].

Table 5. *Results of the determination of the accuracy of cannabidiol.*

No.	Levels of total sample after adding the raw (ng/mg)	Levels of sample before adding the raw (ng/mg)	Levels of analytes added (ng/mg)	*% Recovery*
1	1,89	0,87	1	102
2	1,92	0,86	1	106
3	1,88	0,87	1	101
4	1,87	0,84	1	103
5	1,92	0,87	1	105
6	1,93	0,87	1	106
	Average			103,83

Table 6. *Results of the determination of the accuracy of cannabinol.*

No.	Levels of total sample after adding the raw (ng/mg)	Levels of sample before adding the raw (ng/mg)	Levels of analytes added (ng/mg)	*% Recovery*
1	1,68	0,66	1	102
2	1,68	0,66	1	102
3	1,65	0,64	1	101
4	1,66	0,62	1	104
5	1,69	0,63	1	106
6	1,68	0,67	1	101
	Average			102,67

Table 7. *Results of the determination of the accuracy of Δ^9 THC.*

No.	Levels of total sample after adding the raw (ng/mg)	Levels of sample before adding the raw (ng/mg)	Levels of analytes added (ng/mg)	% Recovery
1	1,63	0,62	1	101
2	1,54	0,64	1	90
3	1,59	0,62	1	97
4	1,62	0,58	1	104
5	1,64	0,59	1	105
6	1,68	0,58	1	110
	Average			101,17

Precision test is done by looping 6 (six) times on samples of cannabidiol, cannabinol, and Δ^9 THC. Retrieved standard of deviation and Relative Standard Deviation (as in Table 8. The value meets the validation requirements for pre cision ie RSD <20%.

Table 8. *Precision Test Results.*

No.	Compound	Level of average (ng/mg)	SD	RSD
1	Cannabidiol	1,90	0,023	1,31%
2	Cannabinol	1,67	0,015	0,90%
3	Δ^9 THC	1,61	0,015	0,90%

Table 9. *Results of linearity test.*

No.	Compound	Regression equation	r
1	Cannabidiol	Y = 443,2x + 8718	0.92
2	Cannabinol	Y = 384,6x + 8886	0,96
3	Δ^9 THC	Y = 275,6x + 9284	0,92

Linearity is determined based on the response of MS standard solution for Cannabinoid (Cannabidiol, cannabinol, and Δ^9 THC). The concentration used was 0.5 ng/mg, 1 ng/mg, 1.5 ng/mg, 2 ng/mg and 2.5 ng/mg, injected into GCMS as 1µL. Based on the calculation of straight line equation and r value are shown in Table 9.

r Values is greater than the value of r tables ($\alpha = 0.05$; n = 5) = 0.88. This shows that the relationship between the concentration of cannabinoid compounds against MS is a linear response significantly. The ability of the tool can be seen from the LOD and LOQ obtained as in Table 10.

Table 10. *Results of LOD and LOQ.*

No.	Compound	LOD (ng/mg)	LOQ (ng/mg)
1	Cannabidiol	0,00017	0,00056
2	Cannabinol	0,00012	0,00039
3	Δ^9 THC	0,00016	0,00054

These results show the GC MS instrument has the ability to be used in the limit concentration of the sample is detected.

4. Conclusion

GC MS technique can be used to analyze the hair of users of narcotic type of marijuana (Cannabis sativa L) within thirty-one minutes. The optimal condition is at a temperature of 500C and extraction by sonication. In the solvent extraction process used sequential is methanol: 2 propanol (1: 1), chloroform, benzene and petroleum respectively for five minutes. Likewise, the optimum levels of extraction can be determined within sixteen minutes. In drug user hair obtained compound cannabidiol, cannabinol, and Δ9THC with concentrations ranging from 0.25 ng/mg up to 2.82 ng/mg.

Acknowledgements

The authors thank Hamonangan Tambunan, Lecturer at the State University of Medan, who helped author.

References

[1] Jakubovic, A., McGeer, E. G., & MsGeer, P. L. (1981). Effect of Cannabinoids and Narcotics on Gonodal Functions. *Neuroend ocrine Regulation and Altered behavior*, 151-168. Doi. 10.100 7/978-1-4684-4067-6_7.

[2] Onaivi, E. S. (2006). *Marijuana and Canabinoid Research*, Method and Protocols, Human Press inc, USA.

[3] Martono dan Jowana. (2006). *Studi Kasus Pemeriksaan Sampel Rambut*, Website: http://www.scribd.com/doc/ 60622481/makalah-studi-kasus.

[4] Balikova, M. (2005). Hair Analysis for Drugs of Abuse, Plausibility of Interpretation, *Biomed Pap Mede Fac Univ Palacky Olomouc Czech Repub*, 149 (2), 199–207. doi.org/10.5507/bp.2005.026.

[5] Indonesian National Narcotics Agency, 2015.

[6] Lestari, S. I (2013). Strategi Badan Narkotika Nasional Kota Samarinda dalam menanggulangi penggunaan narkoba di Kelurahan Sungai Pinang Dalam Kota Samarinda, *Ejournal Ilmu Pemerintahan*, 1(2), 943-955.

[7] Reggra, H. P. (2009). *The Cannabinoid Receptors*, Homana Press, USA.

[8] Khajuria, H. & Nayak, B. P. (2014). Detection of Δ9-tetrahydrocannabinol (THC) in hair using GC–MS. *Egyptian Journal of Forensic Sciences*, 4(1), 17–20. doi.org/10.1016/j.ejfs.2013.10.001.

[9] Starks, M. (1990. *Marijuana Chemistry, Genetic, Processing, and Potency*, Barkeley, California.

[10] Saito, K. (2011). Analysis of drug of abuse in biological specimens. *Journal of Health Science,* 57 (6), 472-487.

[11] Rosani, S. (2013). *Standar Pelayanan Minimal Terapi Medik ketergantungan narkotika, Psikotropika*, dan Bahan adiktif lainnya (Narkoba), BNN, Jakarta.

[12] Swift, W., Wong, A., Li, K. M., Arnold, J. C., & McGregor, I. S. (2013). Analysis of Cannabis Seizures in NSW, Australia: Cannabis Potency and Cannabinoid Profile. *PLoS ONE*, 8(7), e70052. doi: 10.1371/journal.pone.0070052.

[13] Mechoulam, R. (2009). *Cannabinoids*, editor Vincenzo di Marzo, John Wiley and Sons, USA.

[14] Haller, D. L., Acosta, M. C., Lewis, D., Miles, D. R., Schiano, T., Shapiro, P. A., … Newville, H. (2010). Hair Analysis Versus Conventional Methods of Drug Testing in Substance Abusers Seeking Organ Transplantation. *American Journal of Transplantation,* 10(5), 1305–1311. doi: 10.1111/j.1600-6143.2010.03090.x.

[15] Musshoff, F., Junker, H. P., Lachenmeier, D. W., Kroener, L., & Madea, B. (2002). Fully Automated Determination of Cannabinoids in Hair Samples using Headspace Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry. *Journal of Analytical Toxicology*, 26(8), 554–560. doi: 10.1093/jat/26.8.554.

[16] Hegstad, S., Khiabani, H. Z., Kristoffersen, L., Kunoe, N., Lobmaier, P. P., & Christophersen, A. S. (2008). Drug Screening of Hair by Liquid Chromatography-Tandem Mass Spectrometry. *Journal of Analytical Toxicology*, 32(5), 364–372. doi: 10.1093/jat/32.5.364.

[17] Anggraini. (2015). *Tinjauan kriminologi penyalahgunaan narkotika yang dilakukan oleh perempuan di Sungguminasa*, Perpustakaan Pusat Universitas Hasanuddin, Indonesia.

[18] Baumgartner, A. (1979). Radioimmunoassay of Hair for Determining Opiate – Abuse Histories, *The Journal of Nuclear Medicine*, 748–752.

[19] Jones, J., Jones, M., Plate, C., & Lewis, D. (2013). The Detection of THCA Using 2-Dimensional Gas Chromatography-Tandem Mass Spectrometry in Human Fingernail Clippings: Method Validation and Comparison with Head Hair. *AJAC*, 04(10), 1–8. doi: 10.4236/ajac.2013.410a2001.

[20] Engelhart, D. (2014). *Rapid, Robust, and Sensitive Detection of 11-nor-Δ^9-Tetrahydrocannabinol-9-Carboxylic Acid in Hair, Application Note, Forensic/Doping Control*. Agylent Technologies, USA.

[21] Albertini, T., dan Andrea Caruso. (2015). *Determination and Its Main Metabolite Using GC Triple Quadropole Mass Spectrometry*. Thermo Scientific Methode, USA.

[22] Rusevska, K., dan Zoran Zdravkovski. (2011). Simple Extraction Method for Detecting Exogenous Substances in Scalp Hair by GCMS. *Journal of Hygienic Engineering and Design*, Original scientific paper UDC 543.544.3: 543.51, 59–67.

[23] Widayati, D. T. (2008). *Analisis Forensik*, Departemen Narkoba, BNN, Jakarta.

[24] Abdi, K, Abbas Shafiee, Mohsen Amini, Mahmood Ghazi Khansari, dan Omid Sabzevari. (2004). Detection of Morphine in Opioid Abusers Hair by GC/MS. *DARU Journal*. 12(2),. 71–75.

[25] Galand, N., D. Ernour, F. Montigny, J. Dollet, dan J. Pothier. (2004). Separation and Identification of Cannabis Components by Different Planar Chromatography Techniques (TLC, AMD, OPLC). *Journal of Chromatographyc Science*, 42, 130 – 134. doi: 10.1093/chromsci/42.3.130.

[26] Anonimous. (2014). *Rapid, Robust, and Sensitive Detection of 11-nor-Δ^9-Tetrahydrocannabinol-9-Carboxylic Acid in Hair, Application Note, Forensic/Dopng Control*, Agylent Technologies, Inc. USA.

[27] Gouveia, C. A. P. (2011). *Simultaneous Quantification of Morphine and Cocaine in Hair Samples by Gas Chromatography-Mass Spectrometry*, Master Thesis, University Porto, Portugal.

[28] Harmita. (2004). *Petunjuk Pelaksanaan validasi, metode, dan cara perhitungannya*, Majalah Ilmu Kefarmasian, Vol. I, No. 3. Hal. 117 – 135.

[29] Nasser, F. (2007). *Diagnostic Use of Hair Analysis for the Detection of Misuse of Amfetamines and Cannabinoids*, Departement of Forensic Medicine and Science, University of Galgow.

[30] Maunder, et. al. (1974). *An improved procedure for the field testing of cannabis,* Department of Trade and industry, Laboratory of the Government Chemist, UNODC, London.

[31] Kintz, P. (2003). Hair Analysis, Clark's Analysis of Drugs and Poisons, 3rd Edition. Volume 1. Pharmaceutical Press. London.

[32] Sharma, P., MM. Srinivas Bharath, dan Pratima Murthy. (2010). Qualitative high performance thin layer chromatography (HPTLC) analysis of cannabinoids in urine samples of Cannabis abusers, *Indian Journal Med. Res*, 132, 201-208.

[33] Moosmann, B., Roth, N., & Auwärter, V. (2013). Hair analysis for THCA-A, THC and CBN after passive in vivo exposure to marijuana smoke. *Drug Testing and Analysis*, 6(1-2), 119–125. doi: 10.1002/dta.1474.

[34] Grotenhermen, F. (2002). *Cannabis and Cannabinoids*, Editor Ethan Russo MD. The Haworth Integrative Heating Press, Birminghamton.

[35] Florian-Ramrez, N. M., Garzn-Mndez, W. F., & Parada-Alfonso, F. (2012). Gas Chromatography in Forensic Chemistry: Cannabinoids Content in Marijuana Leaves (Cannabis sativa L.) from Colombia. Gas Chromatography - Biochemicals, *Narcotics and Essential Oils*. doi: 10.5772/34503.

[36] Shah, I., Petroczi, A., Uvacsek, M., Ránky, M., & Naughton, D. P. (2014). Hair-based rapid analyses for multiple drugs in forensics and doping: application of dynamic multiple reaction monitoring with LC-MS/MS. *Chemistry Central Journal*, 8(1). doi: 10.1186/s13065-014-0073-0.

[37] Sell, C. (2003). *A Fragrant intoduction to terpenoid Chemistry*, The royal society of chemistry press, Cambridege, United of Kingdom.

[38] Yadav VK, A. S. (2014). Microscopical and Chemical Study of Cannabis sativa. *J Forensic* Res, 05(01). doi: 10.4172/2157-7145.1000210.

[39] Delmifiana, B. (2013). Pengaruh Sonikasi terhadap Struktur dan Morfologi non partikel magnetic yang disintesis dengan Metode Kopresipitasi. *Jurnal Fisika Unand* 2(3), 186-189.

[40] Anonimous. (2003). *Buku Petunjuk Lapangan Pemeriksaan Laboratoris Kriminalistik Barang Bukti Narkotika dan Obat Berbahaya*, Pusat Laboratorium Forensik Bareskrim Polri, Jakarta.

[41] Taufik, M. (2013). *Deteksi Narkotika Jenis Cannabinol dan Morfin dari sampel urine pengguna narkotika*, SNYUbe, Politeknik Negeri Lhokseumawe.

[42] Salomone, A. et al. (2013). Hair analysis as a tool to evaluate the prevalence of synthetic cannabinoids in different populations of drug consumers. *Drug Testing and Analysis*, 6(1-2), 126–134. doi.org/10.1002/dta.1556.

[43] Musshoff, F., & Madea, B. (2006). Review of Biologic Matrices (Urine, Blood, Hair) as Indicators of Recent or Ongoing Cannabis Use. *Therapeutic Drug Monitoring*, 28(2), 155–163. doi: 10.1097/01.ftd.0000197091.07807.22.