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Analysis of Methamphetamine Using Necrophagous Insects For Forensic Entomotoxicology Applications

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Abstract. The high use of methamphetamine (MA) has resulted in many cases of overdose leading to death, where bodies are generally found after several days which complicates the investigation so that a forensic entomotoxicological analysis using insects is carried out. The use of necrophagous insects was chosen because necrophagous insects such as maggots were at the crime scene. Maggot was then analyzed using Gas Chromatography-Mass Spectrometry (GCMS) with several stages. The preliminary test was carried out using the marquis reagent which changed the color of the sample to orange if positive for methamphetamine, then continued with TLC (Thin Layer Chromatography) analysis and obtained the Rf value close to the comparative Rf value, which means that maggot positively contains methamphetamine. Maggot was extracted by maceration and sonication methods using methanol:chloroform 1:3 solvent. Finally, a confirmatory test was carried out using a GCMS and it was found that methamphetamine had a retention time of 3.554 minutes and a peak of 58.1. The results are matched with NIST (National Institute of Standards and Technology) library data. It can be concluded that the use of maggot in analyzing methamphetamine using GCMS can be realized properly.

Keyword: Forensic Entomotoxicology, GCMS, Maggot, Methamphetamine, Necrophagous.

1 Introduction

Drug abuse, especially Narcotics and Dangerous Drugs (drugs) is currently of particular concern because of the high rate of drug abuse in Indonesia. Drug abuse can be one of the factors causing death which has recently increased the finding of abandoned corpses or corpses. Bodies are found within days, weeks to months, making the investigation process more difficult. One of the most important factors in a corpse investigation is determining the cause of death [1]. When human remains are found, the question that inevitably arises is how, when and why this person died [2]. The thing that complicates the process of investigating corpses is the condition of the corpses that

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have decomposed. The decomposition process can obscure toxic chemicals in the body so that the cause of death and time of death (Post Mortem Interval / PMI) will be difficult to determine [3].

Methamphetamine is a strong central nervous system stimulant compound which is a derivative of the amphetamine group [4]. Methamphetamine is listed in group I in the annex to the law of the Republic of Indonesia No. 35 of 2009 concerning narcotics [5]. Methamphetamine is generally available as HCl salt and is known as speed, methamphetamine, and ice, in Indonesia it is known as methamphetamine [6]. The effects of methamphetamine in the short term include increasing activity and euphoria [7]. While the long-term effects are the occurrence of dependence, paranoia, hallucinations and psychosis, mood disorders, impaired motor activity, stroke, weight loss and can also affect the central nervous system strongly which can cause death.

Entomotoxicology is the study of the use of insects as alternative toxicological samples. Insects are a group of animals that have the most orders among all the animals in the world. Insects have the ability to spread to all habitats with various environmental conditions. The ability of insects to survive is carried out by eating various foods such as eating carrion (Necrophagous). Flies (Callophoridae) are an example of necrophagous insects that eat carrion, especially cadaveric corpses, so these insects can be used as a guideline for determining postmortem or time of death. This indicates that insects can be used for medicocriminal investigations studied in forensic entomology [8][9].

Insects that are attracted to corpses can generally be categorized into three groups: necrophagous species that eat corpse tissue, predatory and parasite groups that eat necrophagous insects and omnivore species groups that eat both corpse tissue and other insects. Of these three groups, the necrophagous species group is the most important in helping to estimate the time of death [10].

Due to the large number of cases of death due to drug overdose, this research was carried out and this research was carried out in vitro using the method of taking maggot from rotting meat and identifying it using GCMS.

2 Method

2.1 Maggot Growing

The beef is cut into small pieces, then 250 grams each is put into the tray and the edges of the tray are sprinkled with 5 grams of wheat flour to prevent the maggot from coming out of the tray. Beef is foamed and homogenized with 0.3 gram meitamphetamine in 100 ml water (3000 μg/ml). The tray is covered with the overflowing tray and then overwritten by the container with the weighing

plate and left for eight days in a place where it has not been exposed to rain until it is drained by maggot, can be seen in Figure 1 below.

Figure 1. Maggot flavoring which interferes with metamphetamine in beef

2.2 Maggot Collection

Maggot samples were taken on the third, fourth, fifth, sixth and seventh days (because the eighth day of maggot has changed in shape). The maggot is taken using tweezers and turned off with hot water (> 80 ºC), soaked for 30 seconds [11], then the water is drained. The sample is stored in a sample bottle in the fridge until it is used for further analysis [12].

2.3 Extraction

The maggot was extracted by maceration, the maggot was put into an Erlenmeyer, then it was macerated (soaked) with added dichloromethane (CH_2Cl_2) and then sonicated using an ultrasonic bath for 5 minutes at 42 KHz for the initial washing stage. Discard the organic solution and dry with paper towels. Weigh 1.5 grams of insects and crush the maggot using a mortar. Then add 5 ml of v/v solvent to extract the substance present in the sample. Sonicate for 10 minutes. The solvent used here is 1:3 Methanol and Chloroform. Filtered using Whattman No.42 filter paper and the filtrate was taken, then dried at room temperature. After drying, add 2 ml of methanol again. Then a preliminary test was carried out using marquis reagent, thin layer chromatography and confirmation test with GCMS [13-14]

2.4 Preliminary Test with Marquis Reagent

One drop of maggot extraction results is placed on the drip plate. Then drop 1-2 drops of marquis reagent and observe the color change that is formed. The sample is positive for methamphetamine if the orange color changes to brown. If the sample does not contain methamphetamine, then the reagent does not change color. Marquis reagent consists of a mixture of 95-97% concentrated sulfuric acid and 37% min formaldehyde [15][16].

2.5 TLC Analysis

A TLC plate of silica gel GF 254 with a size of 10 cm x 10 cm was prepared, then heated in an oven at 80 °C for 15 minutes. The test sample was spotted on the plate using a capillary tube 2

cm from the bottom of the plate. The TLC plate was then placed in a chambeir which had been diluted with eluent in eithyl aceitate : ammonia : methanol (8.5 : 0.5 : 1), allowed the eluent to fluctuate up until the elicitation distance was 7 cm. Then the TLC plate was lifted and moved. Then sprayed with iodoplatin, observed stains that appeared and visually were purple in color indicating the presence of methamphetamine. After that, the Rf value was calculated, the result was declared positive if the color of the spot between the sample and the comparison was indicative of each other.

2.6 Confirmation Test with Gass Chromatoghraphy Mass Spektrofotometry (GCMS) Analysis

Maggot was used to confirm the methamphetamine compounds contained in maggot grown on beef. The sample used was 1 µL and injected into the GCMS with the following equipment specifications: GCMS analysis was performed on an Agylent 7890 B GC Instrument and an Agylent 5977A MSD GC Instrument. The column used is DB-5MS, length 30 m, diameter 250 µm, thickness of the stationary phase 0.25 µm. The stationary phase is a mixtureof 5% Diphenyl and 95% Methylpolysiloxan. Injector temperature 250 °C, Interface temperature 290°C, Ion Source temperature 230 °C, Constant flow 1 mL/min, Split 50 : 1, Solven delay 2 min, Mass Scanning 50 to 500. Spitles mode with an injection volume of 1.0 µL. The initial temperature of the oven was held at 100°C for 0 minutes, the temperature increased by 15 °C / minute, until it increased to 280 °C and held for 5 minutes.

3 Results And Discussion

3.1 Maggot Growing

Maggot is grown from fresh beef marinated in the shade with flour around it to prevent the maggot from escaping from the container. Five containers were made for each, one for the control and the other four containers spiked and homogenized with methamphetamine 3000 µg/ml. On the first day of observation, the meat did not change, on the second day the meat smelled and the flies started to come. On the third day, small insects were found starting to growaround under the meat in a damp place (1st instar larvae). On the fourth day of observation, it was found that the insects were quite large (second instar larvae), white insects and had quite active movements. On the fifth day the insects had grown bigger (third instar larvae) and were ready to be picked up. Insects look very active doing feeding activities. The insects found had an average size of 13.6 mm and were milky white in color. The third instar phase lasts until the seventh day of observation where the third instar phase is known to be a phase that requires a longer time than the other instar phases. Insect collection was carried out on the fifth, sixth and seventh day. On the seventhday of observation, the insects look brownish white and are preparing to enter the prepupa phase, then turn into pupaeand then become adult flies again. The results of this observation are not much different from the results of research [17] which obtained the length of instar 1 larvae ranging from

2-3 mm, instar 2 ranging from 4-7 mm, instar 3 rangingfrom 8-13 mm. On the 8th day, there is no Maggot in the meat so that the sampling is only up to the 7th day.

3.2 Maggot Collection

Maggot were taken using forceps and killed with hot water (> 80 ºC), soaked for 30 seconds, then stored in the refrigerator to keep the sample content in it stable. The freezing method is considered better than preservation using alcohol to ensure drug stability and also to reduce the possibility of drug extraction from the matrix when storedin alcohol [18]. Sampling of insects to be analyzed to determine the presence or absence of methamphetamine in insects was carried out on the fifth, sixth and seventh days in accordance with the limitations of the research, namely insects in instar 3 were used as samples in this study and continued with maggot extraction using the sonication method, for example Figure 2.

Figure 2. Maggot

3.3 Extraction by Sonication Method

The initial stage of maggot was added dichloromethane (CH_2Cl_2) and then sonicated to homogenize the solution with maggot, remove the solution and dry it then weigh 1.5 g. This method was sonicated at room temperature for 10 minutes using methanol : chloroform 1:3. Then filtered using Whattman No.42 filter paper and then dried. Drying aims to take the analyte to be analyzed. After drying, the analyte was added with methanol as a solvent and then a preliminary test and confirmation test were carried out by injecting it into the GCMS. Sonication is a method that use sound waves to produce vibrations (change electrical signals into physical vibrations) which can speed up contact between methamphetamine compounds in maggot and solvents even at room temperature [6]. In this study, sonication was used at 42 KHz. The use of two different solvents is based on the principle of "like dissolves like" where polar compounds will dissolve in polar solvents and non-polar compounds dissolve in non-polar compounds. Methamphetamine is a polar compound that will dissolve in polar solutions. So that polar solvents will attract methamphetamine compounds in maggot.

3.4 Preliminary Test with Marquis Reagent

Maggot extraction results, 2 drops are taken and placed on the drip plate. Then drip 1-2 drops of Marquis reagent. Theresults that formed orange color changed to brown which indicated the presence of methamphetamine compounds in maggot . The results obtained can be seen in Figure 3, Table 1 below:

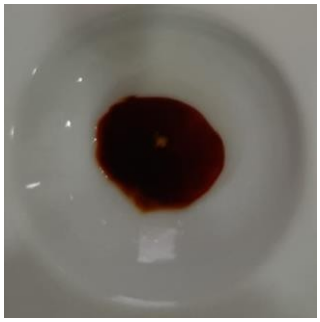

Figure 3. Methamphetamine with marquis reaction

Table. 1. Preliminary Maggot test results with marquis reagent

No.	*Sample*	*Marquis test*	*Informatiion*
1	Comparison sample	*Orange*	Methamphetamine (+++)
2	MA comparison Beef	*Orange*	Methamphetamine (++)

Marquis reagent can give color to compounds containing aromatic rings bonded to C, H, N or O. Methamphetamine with marquis reagent will give a blackish orange or orange color [19]. In this study, the test results obtained a black-orange color, so it was strongly suspected that the sample contained methamphetamine. The color change occurs due to carbonium ions from the marquis reagent which are bound to the aromatic compounds from methamphetamine, causing a color change in the sample.

3.5 TLC Analysis

TLC Analysis was described using solvent eluent ethyl acetate:ammonia:methanol (8.5 : 0.5 : 1). The choice of solvent isdone in such a way as to obtain large amounts of methamphetamine. The results of the analysis showed that the methamphetamine Rf value was 0.648.

3.6 GCMS Analysis

The GCMS analysis was carried out to qualitatively analyze the presence or absence of the methamphetamine compound contained in maggot. After the sample was injected into the GCMS, the Methamphetamine peak was obtained which can be seen in Figure 4,5,6 .

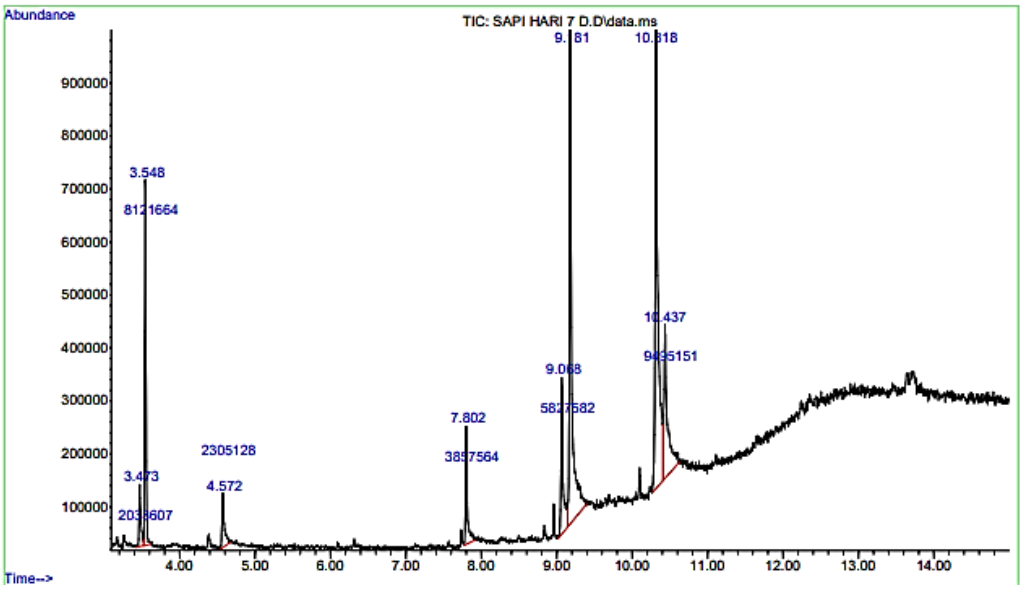

Figure 4 Cromatogram methamphetamine analysis results in maggot on GCM

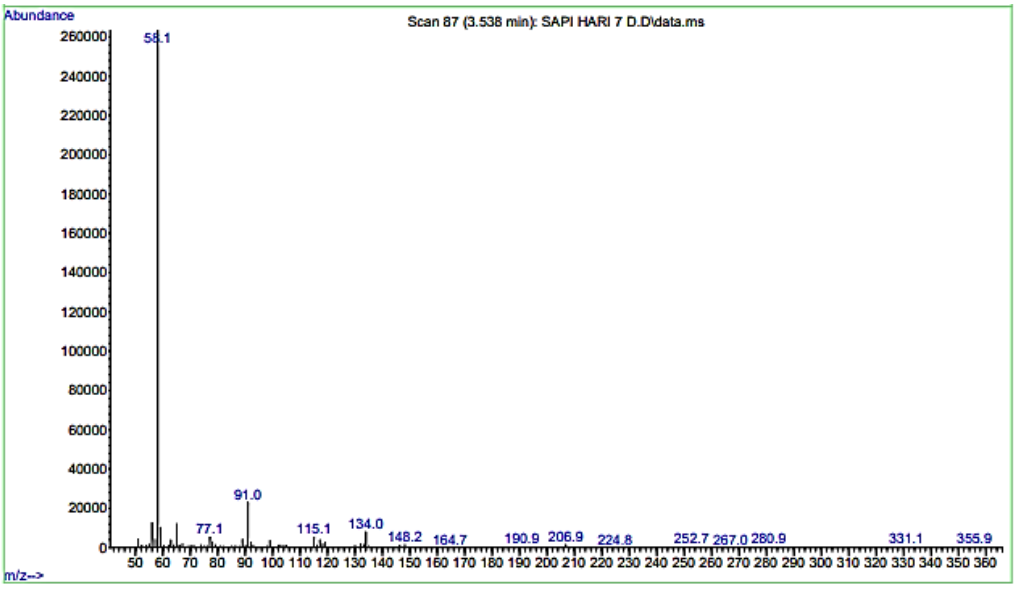

Figure 5 MS spectrum methamphetamine analysis results in maggot on GCMS

Search Libraries: D:\MassHunter\...2 MARET 2018.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - .E

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	3.473	2.55	D:\MassHunter\Library\SWGDRUG 3.2 MARET 2018.L			
			NMP	1747	000872-50-4	42
			4-Chloroamphetamine	1982	000064-12-0	1
2	3.548	10.15	D:\MassHunter\Library\SWGDRUG 3.2 MARET 2018.L			
			Methamphetamine	1834	000537-46-2	83
			N-ethyl-.alpha.methyl-3,4-methylenedioxyphenethylamine	131	000000-00-0	80
			2-Methoxymethamphetamine	1417	000093-30-1	78
3	4.572	2.88	D:\MassHunter\Library\SWGDRUG 3.2 MARET 2018.L			
			Phenylacetic acid methyl ester	464	000101-41-7	35
			Phenibut	1759	001078-21-3	9
			Phenethyl acetate	757	000103-45-7	8
4	7.802	4.82	D:\MassHunter\Library\SWGDRUG 3.2 MARET 2018.L			
			Myristic Acid	648	000544-63-8	93
			Palmitic Acid	729	000057-10-3	45
			Lauric Acid	586	000143-07-7	43
5	9.068	7.29	D:\MassHunter\Library\SWGDRUG 3.2 MARET 2018.L			
			Palmitoleic Acid	221	000373-49-9	95
			Palmitoleic Acid methyl ester	2459	001120-25-8	25
			Pregabalin	690	148553-50-8	8
6	9.181	29.97	D:\MassHunter\Library\SWGDRUG 3.2 MARET 2018.L			
			Palmitic Acid	729	000057-10-3	72
			Stearic Acid	712	000057-11-4	68
			Lauric Acid	586	000143-07-7	59
7	10.318	30.46	D:\MassHunter\Library\SWGDRUG 3.2 MARET 2018.L			
			Elaidic Acid	426	000112-79-8	99
			Oleic Acid	424	000112-80-1	99
			Oleic Acid methyl ester	2484	000112-62-9	2
8	10.437	11.87	D:\MassHunter\Library\SWGDRUG 3.2 MARET 2018.L			
			Myristic Acid	648	000544-63-8	30
			Stearic Acid	712	000057-11-4	22
			Lauric Acid	586	000143-07-7	12

Figure 6 Compounds contained in the maggot sample

The detected methamphetamine MS spectrum in maggot corresponds to the methamphetamine MS spectrum in the GCMS literature. The MS fragmentation pattern of methamphetamine compounds is analyzed as shown in Figure 7 below

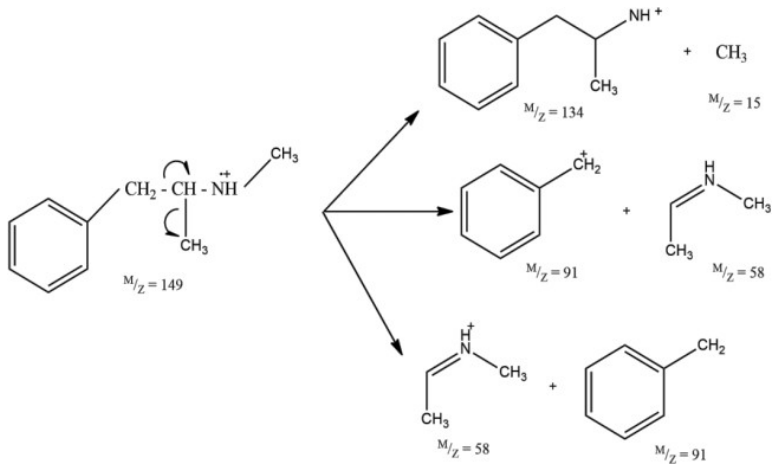

Figure 7. MS Fragmentation of Methamphetamine Compounds

Based on the GCMS chromatogram results, the methamphetamine compound was detected at a retention time of 3.548 minutes with an area of 8121664. The results showed that there were several other peaks besides methamphetamine, which means other compounds were found in maggot. In the MS spectra, MA has a fragmentation pattern of m/z peaks of 134.0, 91.0, and 58.1. The highest peak is at m/z 58.1 which is referred to as the peak base and is usually assessed as 100% compared to the height of the other peaks. The peaks obtained correspond to the ion fractions that methamphetamine has, namely 58, 91, 134, this proves that the compound being analyzed has a fragmentation pattern identical to the ion fraction of methamphetamine according to NIST (National Institute of Standards and Technology) library data. This makes it certain that what is being analyzed is a methamphetamine compound.

4. Conclusion

Based on the results of the data obtained from the chromatogram on the GC and MS spectra, the data matched with the NIST (National Institute of Standards and Technology) library data. With a retention time of 3.548 minutes, an area of 8121664 and a peak of 58.1 on methamphetamine. So it can be concluded that Methamphetamine content can be well analyzed on the GCMS instrument from grown maggot. This proves that forensic entomotoxicology using maggot as a sample can be used in analyzing the causes of death in decomposing corpses due to the abuse of methamphetamine-type drugs.

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