

Development of sonication and gcms methods for analysis of methamphetamine levels in the shabu-shabu hair using

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Abstract. Hair can be used as an option in analyzing the levels of methamphetamine in the hair of shabu-shabu users. The research aimed to find out the optimum solvent was used in the extraction of sonication method in qualitative analysis of methamphetamine in the hair of shabu-shabu users, and to determine the levels of methamphetamine in the hair of shabu-shabu users from sonication-based analytes using GCMS. Hair samples of drug users of methamphetamine were collected from the Rehabilitation Center of Datuk Etam - Tj. Morawa. The hair collected is the user's hair after 0-14 days, 30 days, 150 days, 210 days after narcotics use. The results showed that methamphetamine could be detected in hair specimens with a marquis test which gave a brownish yellow discoloration. The optimum solvent was used in the extraction of sonication method in the qualitative analysis of methamphetamine in hair using methamphetamine: acetone: ammonia. Levels of methamphetamine in the hair of shabu-shabu users from sonication-based analytes by means of injecting 1 μ L into GCMS in the hair sample of user 1 on day 0-14 = 1.10; day 60 = 1.03; day 150 = 0.93; Day 210 = 0.71. 2nd user on day 0-14 = 1.04; day 60 = 1.00; day 150 = 0.88; Day 210 = 0.71. 3rd user on day 0-14 = 1.09; day 60 = 1.01; day 150 = 0.81; day 210 = 0.73. 4th user on day 0-14 = 1.00; day 60 = 0.92; day 150 = 0.86; day 210 = 0.69. 5th user on day 0-14 = 1.02; day 60 = 0.89; day 150 = 0.77; day 210 = 0.64.

1. INTRODUCTION

Methamphetamine is a class 2 psychotropic drug which is a stimulant that can strongly influence the central nervous system which causes additive effects when consumed. In North Sumatra, listed 288.226 life with 115 290 Life users of crystal meth. According to the latest data from Badan Narkotika Nasional (BNN) 2012 [1], the type of drug that is most widely used by addicts who receive therapy and rehabilitation services is shabu-shabu/methamphetamine as many as 4,697 people [9].

Methamphetamine, C₁₀H₁₃N, has a molecular weight of 149.23, a central nervous stimulant. Methamphetamine is generally available in the form of HCl salt, and is called speed, meth, ice. Also known as "crank and crystal" [6]

The presence of several types of drugs in the body can be analyzed through body fluids such as urine and blood, as well as non-bodily fluids such as hair. Excess use of hair samples rather than urine and blood to analyze drugs is having hair information on the presence of older drugs with a range of weeks to months compared to urine or blood that only detects with a time range of several hours to several days [5].

Sonication is one liquid-liquid extraction method that utilizes ultrasonic waves with a frequency of 42 kHz which can accelerate the contact time between samples and solvents even at room temperature. (Ashley, 2001). Sonication extraction methods are also efficient and shorten the extraction time [7].

Gas Chromatography - Mass Spectrometry (GC-MS) is capable of detecting drug levels / shabu-shabu with a concentration of less than 1 µg/L and requires a relatively short processing time. [10].

To prove someone using drugs (users) samples are taken in the form of body fluids or not body fluids such as urine, blood, sweat, saliva and hair. Choosing a hair sample is preferred because of the length of detection that we can do in the span of weeks to months when compared to urine and blood with a shorter time in the urine sample 1x24 hours and blood can be analyzed 2x24 hours. Examination of drug users through hair will provide an analytical impetus with the development of sensitive and rapid techniques to meet the needs of drug abuse detectors which can also show the history of narcotics use. This will answer some of the clinical problems of toxicology and forensics in drug abuse [2].

Purification process is needed in the analysis to give good results without losing too much analyte. Purification procedure is very important because in the incorporated hair sample several other substances must be separated first until the substance to be detected is pure. Cut 1 cm hair is washed so that the impurities are wasted then with organic solvents in sonication. The purpose of sonication is to ensure the solvent solution enters the hair through the follicle [3].

This study aims to determine the effect of the optimum solvent system used in the extraction of sonication methods in qualitative analysis of methamphetamine in hair methamphetamine users, and to determine the levels of methamphetamine in the hair of methamphetamine users from sonication analytes using GCMS.

2. METHODS

Material

The sample used in this study is a hair sample. The chemicals used in this study were chemicals in pro analysis degree (pa) consisting of chloroform, ethyl acetate ($\text{CH}_3\text{COOC}_2\text{H}_5$), methanol (CH_3OH), glacial acetic acid, aeton, ammonia, formalaldehyde 37%, concentrated sulfuric acid and distilled water.

Tools

The tools used include glassware commonly used in analytical laboratories, - Ultrasonicbath, and spot preparation. Hair samples were carried out at the Analytical Chemistry Laboratory USU – Medan Indonesia, and GCMS Analysis was carried out in the Laboratory of the Technical Services Section of the Field of Testing and Field Goods Identification (BPIB) Belawan - Medan, indonesia.

2.1. Making the Marquis Reagent

Marquist reagent consists of two reagents, the first reagent was made by mixing as much

as 8-10 drops of 37% formaldehyde into glacial acetic acid and the second reagent was concentrated sulfuric acid.

2.2. Hair Specimen Preparation and Analysis

As many 30-40 mg of hair using methamphetamine is mashed using alu and lumpang. Then washed using methanol 3 times each for 5 minutes. Subsequently, it was syndicated using a solvent system, namely methanol: acetone: ammonia, ethiasetate: methanol: ammonia and chloroform: methanol: acetic acid with a ratio of 5: 1.2: 0.08, 8.5: 1: 0.5 and 7.5: 2: 0.5 at room temperature for 30 minutes. Next, it is recycled with chloroform for 5 minutes. Liquid-liquid extraction using methanol. Cooled at room temperature. Filtered using filter paper. Filtrate was identified using Marquist reagents. Observed the color changes that occur. As much as 1 μ of sonication analyte is injected into GCMS.

3. RESULT AND DISCUSSION

3.1. Qualitative Test of Savu-Sulfur Crystals

15 mg of powder dissolved with 3.0 mL of ethanol. This solution was extracted by shaking 3000 rpm for 6 minutes with 2 mL standard solvent (Toxitube® Variant). Then the crystal methamphetamine solution was made into 10 ppm, 50 ppm and 100 ppm. Then the organic phase is taken and then injected into the GCMS instrument.

Table 1. Qualitative Test Results of Crystal Methamphetamine

Sample	Marquis Preliminary Test	Information
Crystal Methamphetamine	Brownish yellow	Methamphetamine (++++)

3.2. Confirmation of Crystal Methamphetamine Sample Test Using GCMS Technique

Confirmation tests are carried out to ascertain the methamphetamine compounds in the sample and qualitatively determine what compounds are in them. As much as 1 μ L of crystal methamphetamine samples were injected into the GCMS instrument obtained by GC chromatogram which can be seen in Figure 1.

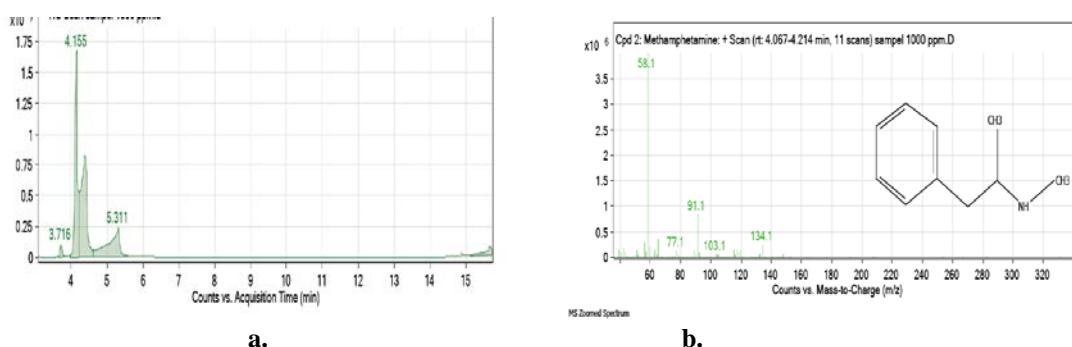


Figure 1. a. Methamphetamine Chromatogram in Crystals Methamphetamine b. MS Methamphetamine Spectrum

3.3. Qualification Test for Sonication Filtrates

Qualitative Test of Filtration of Sonication Results with Comparison of Solvent Systems using the Marquist Reagent can be seen in Table 2.

Table 2. Qualitative Test Results of the Sonication Result Filtrate

NO	Hair Samples	Marquish Test Preliminary Test											
		Sonication Method with Solvent Comparison											
		Methanol: Acetone: Ammonia (1:1:1)				Ethylacetate: Methanol: Ammonia (1: 1: 1)				Chloroform: Methanol: Acetic Acid (1: 1: 1)			
		Days to				Days to				Days to			
		0-14	30	150	210	0-14	30	150	210	0-14	30	150	210
1	User 1	++	++	++	++	+	+	+	+	+	+	+	+
2	User 2	++	++	++	++	+	+	+	+	+	+	+	+
3	User 3	++	++	++	++	+	+	+	+	+	+	+	+
4	User 4	++	++	++	++	+	+	+	+	+	+	+	+
5	User 5	++	++	++	++	+	+	+	+	+	+	+	+

Information:

++: brownish yellow color is very clear

+: brownish yellow color is quite clear

Analysis of narcotic compounds using hair samples has a very important role in the field of forensic toxicology against the history of drug abuse. Hair samples taken are hair that is located on the back of the head and which is close to the scalp. The main reason for choosing a hair as a sample is that the hair has the ability to absorb exogenous substances and these substances will remain unchanged for several years.

Methamphetamine can be detected a few months after last consumption, because these drugs enter the hair root through the capillary and will be embedded in the hair shaft needed special treatment to be able to remove it, the method used in this study is sonication.

Methamphetamine is a polar compound and methamphetamine will dissolve in polar solvents. After being authenticated, the sonication of the filtrate was extracted using methanol. The goal is to attract methamphetamine compounds that have come out of the hair. After obtaining concentrated extract containing methamphetamine, a qualitative test was performed using marquish reagent which will produce a brownish yellow color. From the results of qualitative tests with 3 (three) solvent system comparisons, the colors produced have different intensities. Good results are shown in methanol: acetone: ammonia solvent system can be seen in Table 3.

3.4. Determination of Methamphetamine Compounds in Shabu-Shabu Hair Using GCMS Technique

In this study obtained positive (+) methamphetamine results in all hair samples ranging from 0 days to 210 days after consuming methamphetamine can be seen in the table 3.

Table 3. Methamphetamine Compounds in Shabu-Shabu Hair

NO	Hair Samples	Compound Methamphetamine (ng/mg)			
		Analysis of Sonication Results with Solvent Comparison			
		Metanol:Aseton:Amonia (1:1:1)			
		Days to			
		0 – 14	60	150	210
1	User 1	1,10	1,03	0,93	0,71
2	User 2	1,04	1,00	0,88	0,71
3	User 3	1,09	1,01	0,81	0,73
4	User 4	1,00	0,92	0,86	0,69
5	User a 5	1,02	0,89	0,77	0,64

Hair growth on average 0.6-1.42 cm per month (Saitoh, 1969) while the hair size of each user of methamphetamine is also relatively long ranging from 5 cm to 21 cm, if associated with hair growth it can be known that the average user is 12 months old so that it is possible to use methamphetamine compounds that have been consumed in the hair of the shabu-shabu user but with a very small concentration so that they can only be read by GC-MS.

According to Kintz (2000) the advantages of hair tests compared to urine are that the hair has information on the presence of longer drugs with a range of weeks to months compared to urine or blood which only detects with a range of hours to several days. This also supports the findings obtained from this study. Thus it can be ascertained that the results obtained are methamphetamine stored in the hair for a long period of time or the results of regular consumption. The results of methamphetamine analysis in the hair samples of shabu-shabu users using the SIM method are shown in Table 3.

3.5. Method Validation

Validation of methods according to the United States Pharmacopeia (USP) is carried out to ensure that the analytical method is accurate, specific, reproducible, and resistant to the range of analytes to be analyzed. The method validation of the GCMS technique is carried out through accuracy (accuracy) test with percent recovery (% recovery), linerity, and limit of detection and limit of quantification.

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1. Accuracy Test Results (Accuracy) with Percent Returns (% Recovery)

This research was carried out a recovery test or% recovery with addition method. This method is by adding a number of standard methamphetamine solutions with a concentration of 2 ng / mg into a hair sample of shabu-shabu users in patient 1 after 14 days of use, 3 replications are made. 0.1µl was injected into GCMS. The average% recovery result = 104, this value has met% recovery is 70-120% [4].

2. Precision

Precision tests were carried out on crystal methamphetamine (methamphetamine) samples with a loop 3 (three) times, determined based on the Relative Deviation Standard (SDR). Standard deviation and relative standard deviation (RSD) are obtained in table 4.

Table 4. Value SD and RD Values

Compound	Average Level (ng / mg)	SD	RSD
Mhetamphetamine	6,61	0,0775	1,1725 %

3. Linerity

Linerity is determined based on the response of MS to standard solutions. The methamphetamine standard solution was determined to be 0.1 ng / mg, 0.5 ng / mg and 1 ng / mg. Injected into GCMS as much as 1 Ml. Straight line equations and r values are calculated using SPSS version 13 and the final results can be seen in Table 5.

Table 5. Linear Values

Compound	Regression Equations	r
Mhetamphetamine	$Y = 1E + 56x - 2E + 06$	0,968

Based on Table 5 the price of $r = 0.968$. The value of r is greater than the value of r table ($\alpha = 0.05$; $n - 5$) = 0.08. Based on these data shows that the relationship between the concentration of metamfetamn compounds to MS response is linear.

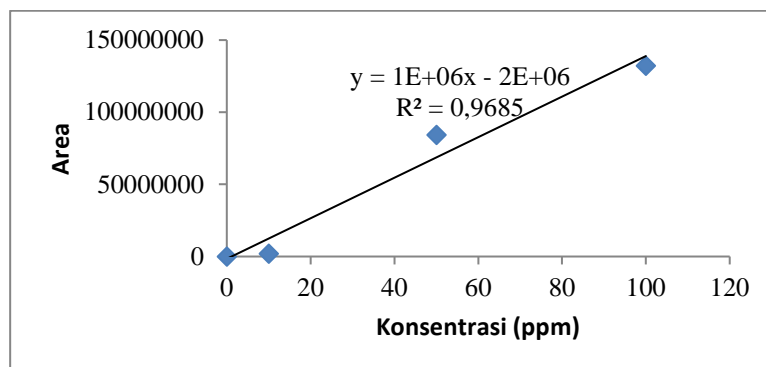


Figure 2. Standars Methamphetamine Regression Curve

4. Limit of Detection and Limit of Determination (Limit of Quantification)

Table 6. LOD and LOQ values

Compound	LOD (ng/mg)	LOQ (ng/mg)
Mhetamphetamine	0,00000256	0,000000775

Based on LOD and LOQ data, it can be seen that the ability of GCMS instrumentation that has been used and the limit of sample concentration that can be detected. Testing of crystal methamphetamine and hair samples from narcotics users is still carried out on a laboratory scale. The method developed requires further testing at an external laboratory so that this method gets further recognition from the external to be used as a standard method.

4. CONCLUSION

1. The optimum solvent was used in the extraction of sonication method in the qualitative analysis of methamphetamine in hair using methamphetamine: acetone: ammonia.
2. Levels of methamphetamine in the hair of shabu-shabu users from sonication-based analytes by means of injecting 1 μ L into GCMS in the hair sample of user 1 on day 0-14 = 1.10; day 60 = 1.03; day 150 = 0.93; Day 210 = 0.71. 2nd user on day 0-14 = 1.04; day 60 = 1.00; day 150 = 0.88; Day 210 = 0.71. 3rd user on day 0-14 = 1.09; day 60 = 1.01; day 150 = 0.81; day 210 = 0.73. 4th user on day 0-14 = 1.00; day 60 = 0.92; day 150 = 0.86; day 210 = 0.69. 5th user on day 0-14 = 1.02; day 60 = 0.89; day 150 = 0.77; day 210 = 0.64.

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