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Article 758 : Development and Validation GC/MS Method for Methamphetamine Analysis in Urine by Miniaturization QuEChERS

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Review:Development and Validation GC/MS Method for Methamphetamine Analysis in Urine by Miniaturization QuEChERS

Request for Review

Article Title

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Development and Validation GC/MS Method for Methamphetamine Analysis in Urine by Miniaturization QuEChERS

Abstract

In the present study, we describe the development of fast and simple gas chromatography (GC) method for determining methamphetamine in the urine of drugs abuser. For this study, a gas chromatography equipped with mass spectroscopy and capillary column TG-5SILMS (5% phenyl methyl siloxane, 30m x 0.32 x 0.25µm) was used the carrier gas flow rate at 1.0~~ml~~mLmL/min, the temperature inlet and detector set at 300°C and the oven temperature was programmed to initiate at 50°C and held for 1.5 minutes, the temperature was raised to 300°C at a rate 40°C/min and held for 3 minutes. Sample pre-treatment by modification of the QuEChERS method includes using a relatively large amount of inorganic salt, extraction volume and extraction cycle. Combining 160mg magnesium sulfate, 40mg sodium chloride and ~~400µl~~ 400µL acetonitrile as organic solvent provided the optimum condition for processing a 400µL~~l~~ urine sample. The validation test found that the detection limit for methamphetamine was 0.36µg/~~m~~mL~~l~~, the quantitation limit was 1.09µg/~~ml~~mLmL, the calibration curve was linear with the regression line y=1.0489x-3.7914, coefficient (r) was 0.9973. The recovery of the analyte spiked into urine at 5, 7 and 9µg/~~ml~~mLmL on average was 100.5±2,33% for intraday dan 93.3±7.21% for inter-day. The precision was good, with a coefficient of variation on average was 2.31%. The method was applied to 4 urine of drugs abuser in which the first abuser (25.51µg/~~m~~mL~~l~~), the second abuser (15.05µg/~~ml~~mLmL), the third abuser (17.72µg/~~ml~~mLmL) and the last abuser (3.08µg/~~m~~mL~~l~~) were successfully quantitated.

Keywords: *GC-MS; QuEChERS; urine; methamphetamine; validation.*

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1. Introduction

The United Nations Office on Drugs and Crime (UNODC) as a world body dealing with narcotics issues notes that at least 271 million people worldwide or 5.5% of the total global population of the world's population with an age range between 15 to 64 years have consumed drugs, at least the person had consumed narcotics in 2017. At the end of 2019, Indonesia's population reached ±271 million people, of which 3.41 million people or around 1.80% were drug

abusers (BNN et al., 2019). The use and abuse of methamphetamine have been on the rise for a decade. Based on data from the Criminal Investigation Agency of the Republic of Indonesia Police, in 2019, the distribution of methamphetamine-type drugs reached 2.7 tons, and in 2020 it increased by 119% to 5.91 tons (Dalimunthe et al., 2015).

Methamphetamine is an illegal drug that is very dangerous and damaging. The active compound in methamphetamine can stimulate the Central Nervous System (CNS), so its distribution is prohibited in Indonesia, so the government takes this matter seriously by issuing Law Number 35 of 2009 concerning Narcotics as a legal basis that the distribution and abuse of narcotics is an activity that is against the law, which is determined as a crime. Methamphetamine is a member of the amphetamine class in which the amino group (S)-amphetamine carries a methyl substituent. It has roles as a neurotoxin, psychotropic drug, central nervous system stimulant, xenobiotic and environmental contaminant (Rothman et al., 2001).

Drug compounds can be monitored through body fluids such as urine, sweat, saliva, and blood. Methamphetamine is excreted in the urine about 70% of the dose within 24 hours, 30-50% as methamphetamine, and 10-15% as its metabolite. Metabolites of methamphetamine in urine are amphetamine and 4-hydroxy methamphetamine (Cruickshank & Dyer, 2009; Kim et al., 2004). The percentage of parent methamphetamine in the urine is large enough so that a methamphetamine test using a urine sample can be performed. In addition, urine is easy to obtain and does not require expertise to get it.

Before carrying out an analysis of the concentration of methamphetamine in urine samples using sophisticated inspection techniques such as gas chromatography, the process of purifying methamphetamine from urine samples is a process that must be followed. The extraction and purification of analytes are important in determining drugs and metabolites in biological samples. Traditional sample extraction or purification methods such as liquid-liquid extraction (LLE) or solid phase extraction (SPE) consume ~~a lot of~~much time, have many steps and are quite complicated, require a variety of chemicals and in large enough quantities, the risk loss of analyte or contamination higher and not quite safe for the environment because the waste produced is quite high (Campêlo et al., 2021; Correia-sá et al., 2018; Samanidou, 2018; Stevens et al., n.d.; Westland & Dorman, 2013). Another problem also comes from the biological sample itself, where the concentration of methamphetamine in the urine is very small (trace analyte), and the biological sample has a very complex matrix.

In 2003 Anastassiades, et.al., introduced the QuEChERS (quick, easy, cheap, effective, rugged and safe) method for analyzsing pesticide residues in fruits and vegetables. Through QuEChERS, the previously complicated method can be simplified into two easy steps; the first stage through liquid-liquid extraction and the second stage through the solid phase. Further analysis was carried out using gas or high-performance liquid chromatography (Fanning & Searfoss, 2017). The QuEChERS method is similar to LLE but highly selective, like SPE. The QuEChERS method is based on extraction with acetonitrile or ethyl acetate solvents and dehydration in the presence of salts such as magnesium sulfate and sodium chloride (Majid et al., 2017).

Several studies have been carried out to modify the QuEChERS method by Fanning et al., where they reduced the use of magnesium sulfate and sodium chloride salts from the previously commonly used 4g magnesium sulfate:1 gram sodium chloride to 800mg:200mg due to the use of the sample, which is also less. Likewise, with the use of acetonitrile which is reduced proportionally. From the results of this study, the recovery results were quite good, namely an average of 81% and 83% from two different analyte concentrations and the average of various types of drugs in beef liver samples (Fanning & Searfoss, 2017). Majid et.al., carried out another study. They used 400mg of magnesium sulfate and 200mg of sodium chloride with a volume of 1mLmL of the urine sample and added buffer until the sample pH became 8-9. This study produced a fairly good recovery value of 78% (Majid et al., 2017).

Several studies have been carried out to modify and optimize the QuEChERS method to improve the QuEChERS method by optimizing the use of solvents and partition salts depending on the target analyte to be analyzed, besides that modifications were also made for the use of sorbents as clean-ups and finally through miniaturization of QuEChERS. The various QuEChERS modifications aim to increase extraction effectiveness, reducing the influence of the sample matrix and increasing selectivity, specificity and sensitivity (Schmidt & Snow, 2016).

The QuEChERS modification is also very important, especially in cases where the number of samples available is small, and is very popular in analytical chemistry because of its advantages such as reduced cost of solvent, amount of salt and sorbent, easier handling, processing, and a minimal amount of waste disposal compared to classic extraction procedures. This feature enables higher throughput analysis with the consequent increase in accuracy and a significant reduction in time and cost. The development of the QuEChERS method pushes the challenge of miniaturization and automation even further (Perestrelo et al., 2019). Several studies in the forensic field have

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been carried out to modify QuEChERS through miniaturization of QuEChERS, but the derivatization and evaporation stages are still being carried out (Alves et al., 2017); (Pouliopoulos et al., 2018) and (Matsuta et al., 2013).

Based on the description above, it is necessary to conduct research related to modifying QuEChERS through miniaturization of QuEChERS (m-QuEChERS) ~~for the extraction of~~to extract methamphetamine in urine before being analyzed using gas chromatography-mass spectroscopy. Compared to the previous existing research, the novelty in this study is simplifying the extraction process by not carrying out the derivatization step but still considering the selectivity, specificity, sensitivity, accuracy and precision of an analytical method.

2. Experimental Section

2.1. Materials

2.1.1. Reagents and Materials

Acetonitrile, MgSO4, NaCl and potassium carbonate were purchased from Merck Indonesia. The standard methamphetamine hydrochloride 1000µg/~~ml~~mL in methanol was purchased from Cayman Chemical. The Internal standard (caffeine solution 1000µg/~~ml~~mL in methanol) was purchased from Supelco (USA); the other reagents and solvents used were of analytical grade or better quality. Deionized water from the Mili-Q gradient system (Millipore). Disposable 2~~m~~mL~~l~~ safe-lock test tube and 15~~ml~~mLµµ tube with screw cap (Eppendorf, Germany).

2.1.2. Urine samples

The sample to be used in this study ~~is~~ was random urine. Negative urine for methamphetamine was obtained from volunteers (laboratory staff) who had not taken any medication in the past month.

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2.2. Methods

2.2.1. Sample preparation method

Magnesium sulphate and NaCl were each pulverized and mixed well at a weight ratio ~~of~~ 4:1. The mixture (200mg each, 5mg) was weighed into the disposable 2~~ml~~mL safe lock test tubes. A 400µ~~u~~l volume acetonitrile was added into disposable 2~~ml~~mL safe lock test tubes containing the mixture of MgSO4 and NaCl. A 400µ~~u~~l urine sample was adjusted to pH over 10 using K2CO3 buffer and added to the test tube. Immediately vortex-mixed for 1 minute, followed by centrifugation for 5 minutes at 10.000RPM using a high-speed refrigeration centrifuge MPW-150 (Med. Instrument, Polandia). The organic phase was separated using a pipette. In order to recover the organic extract contained in the cake of an inorganic salt, an additional 400µ~~u~~l acetonitrile was added into the test tube and the organic phase was sampled after vortex and decantation, do this step twice. These organic extracts were combined and evaporated under a gentle nitrogen stream. The residue obtained was dissolved in 400µ~~u~~lL of acetonitrile.

2.2.2. GC-MS

Sample injection was done manually with a volume of 2µL. The analyte was separated using a TraceGold TG-5SILMS capillary column (0.25mm. id, 30m, 0.25µm with 5m safeguard), and the mobile phase was helium (purity ≥99.999%). The flow rate of the carrier gas is 1~~mL~~mL/min constantly by the system, splitless injection mode. The oven temperature was programmed to follow the CoA reference, which was 50°C for 1 minute, then increased at 40°C/min until it reached 300°C. At the end of the analysis, the conditions were set at 300°C for 3 minutes to eliminate the effects of impurities from the sample. Injector temperature and MS transfer line temperature ~~are~~ were set at 300ºC. The MS ionization system use~~d~~s Electron Impact (EI) with a strength of 70eV at 300ºC. The Thermo Scientific Chromeleon Chromatography Data System (CDS) software use~~d~~sd for data processing and operational systems.

3. Results and Discussion

3.1. Optimization of GC-MS Conditions

Optimization of conditions ~~is~~ was carried out by adjusting the rate of increase in oven temperature, gas flow rate, injection mode and sample volume. The optimization result parameters used for optimization evaluation in several gas chromatographic conditions include relative retention time, resolution, theoretical plate number, match factor, and run time. Complete gas

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chromatography optimization results can be seen in Table 1.

Table 1. Results of optimizing the determination of methamphetamine levels with IS caffeine in several gas chromatography conditions

Method	Optimization Parameters				Results					
	Flow Rate (~~ml~~mL/min)	Temperature Rising Speed	Injection Mode	Sample Volume (µL~~l~~)	Relative Retention Time met/IS (min)	Resolution met/IS*	Theor~~e~~itical Plate Numbers met./IS	Relative Area met/IS*	SI* met	*Run Time (min)*
1	1	40°C/min	Splitless	1	1,~~,~~38	3.85/2.06	254524/ 1381146	1,~~,~~24	885	11.21
2	1.5	40°C/min	Splitless	2	1~~,~~,39	5.08/1.88	289549/ 1268266	1,~~,~~33	895	11.21
3	1,~~,~~5	30°C/min	Splitless	2	1,~~,~~49	3.53/4.55	123300 / 2114975	2,~~,~~16	915	13.31

*SI = *match factor;* met = methamphetamine; IS = Internal Standard (Caffeine)

From Table 1, the retention time (t_R) of methamphetamine was 5.095 minutes, and IS was 7.020 for method 1. Method 2 showed methamphetamine's t_R was 5.064 minutes and IS ~~-~~7.023, while method 3 showed methamphetamine's t_R was 5.390 minutes and IS 7.993. The results of gas chromatography optimization ~~are~~ were assessed from several parameters, namely resolution, theoretical plate number and match factor.

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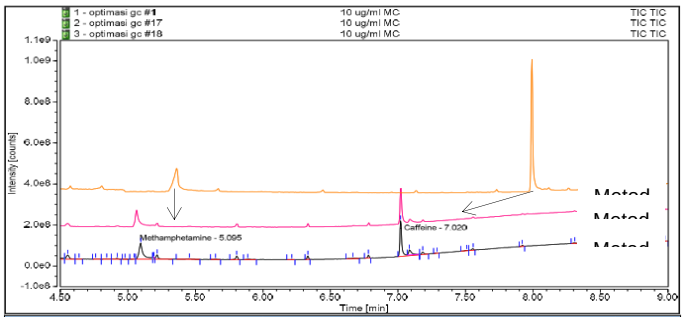

Figure 1. Overlay 10 µg/~~ml~~mL methamphetamine chromatogram method 1, ~~method~~ 2, and ~~method~~ 3 in acetonitrile using gas chromatography ~~method~~.

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The result of optimizing the selected gas chromatography conditions ~~is~~ was method 1, namely an initial temperature of 50°C for 1 minute with a temperature increase of 40°C/minute until it reache~~d~~s 300°C, which is maintained for 3 minutes. The inlet temperature ~~is~~ was 300°C, the splitless mode and the gas fl~~o~~ow rate is 1.0 ~~ml~~mL/minute. Method 1 was chosen because it has several advantages compared to methods 2 and 3; among others, the gas flow rate and sample

volume ~~are~~ were less than methods 2 and 3, namely 1~~ml~~mL/min, with a shorter run time and faster retention time of analyte and IS, but still shows the same optimization results as both methods 2 and 3. Where the optimization results showed that the three methods have a resolution value greater than 1.5, the theoretical plate number ~~is~~ was greater than 10,000, and the match factor (SI) value ~~is~~ was greater than 800.

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3.2. Optimization of Extraction Method

The selection of inorganic salts in the modification of QuEChERS in this study was based on the original method of QuECHERS, which used MgSO4 and NaCl with a ratio of 4:1, and the balance of sample volume to solvent was 1:1(Schmidt & Snow, 2016). Extraction optimization was carried out by modifying several extraction parameters, namely the amount of inorganic salts, extraction volume and extraction cycle. Differences in variation can be seen in S1.

Table 2. Variation of methamphetamine extraction volume with inorganic salt composition: urine volume: acetonitrile = 1:2:2

Sample ID	Weight of Inorganic Salt* (mg)	Urine Volume (µ~~l~~L)	~~Asetonitril~~Acetonitrile Volume (µ~~l~~L)	Ex~~k~~straction Volume (µ~~l~~L)	Extraction Cycle
Extraction (E1)	50	100	100	200	1 2 3
Extraction (E2)	100	200	200	400	1 2 3
Extraction (E3)	200	400	400	800	1 2 3
Extraction (E4)	300	600	600	1200	1 2 3

*Inorganic Salt = $MgSO_4$:NaCl (4:1)

The results of standard extraction of 10 µg/~~ml~~mL methamphetamine in urine with several variations in extraction volume can be seen in Table 3, where the relative area ~~is~~ was obtained from the ratio of the methamphetamine area to the IS area, then %CV and %Recovery (%R) was~~is~~ calculated (Campêlo et al., 2021). The recovery percentage ~~is~~ was calculated from the ratio of the relative area of the analyte in the spiked sample after extraction to the relative area of the standard at the same concentration (Orfanidis et al., 2022). From Table 3, it can be seen that for the E1 method, injection into the gas chromatography was not carried out because it was difficult to

separate the organic phase from the aqueous phase because the extraction volume was very small, namely 100µl, so the analysis results were not obtained. Extraction method 2 with 1 extraction cycle was also not analyzed using gas chromatography because the organic phase produced was cloudy after the evaporation and restitution process using acetonitrile, which was feared would clog the gas chromatography column.

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Table 3. Optimization results of methamphetamine extraction, mean relative area of methamphetamine to IS (n=3), Recovery ± SD (%) and Coefficiency of Variation (%CV).

Method	Extraction cycle (kali)	Visual	Mean Relative Area met./IS	*Recovery* ± SD (%)	%CV
E1	1	The organic phase layers are difficult to separate			
	2				
	3				
E2	1	Cloudy organic phase			
	2	Clear organic phase	0,24	12,28 ± 1,10	8,92
	3		0,31	15,57 ± 0,60	3,85
E3	1	Clear organic phase	0,58	28,96 ± 1,94	6,70
	2		0,67	33,46 ± 4,78	14,3
	3		2,01	101,2 ± 2,30	2,27
E4	1	Clear organic phase	1,59	79,89 ± 12,89	16,14
	2		1,69	85,35 ± 5,28	6,19
	3		1,77	88,83 ± 6,17	6,95

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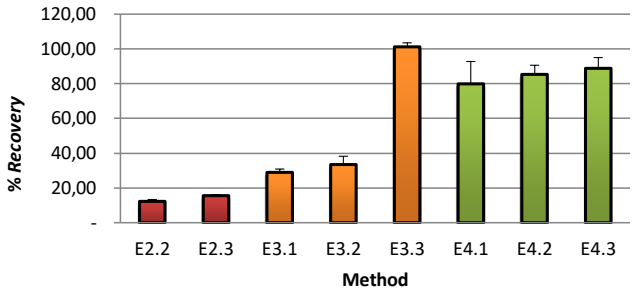

Figure 2. Methamphetamine recovery diagram by the m-QuEChERS method.

It can be seen in Figure 2 that the extraction that gives the best % recovery results is the E3 extraction volume (800µl) for 3 extraction cycles with a %R of 101.2 ± 2.30% and a %CV of 2.27%. ~~Thus t~~The next stage of extraction ~~is~~ was carried out using these conditions.

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3.3. Qualitative Test of Methamphetamine in Urine Samples by Gas Chromatography-Mass Spectroscopy

The two chromatogram images (figure 3,4) showed that the standard methamphetamine retention time is 5.016 minutes, and in the urine sample is 5.153 minutes. The relative retention time for the solution was 1.39, and the methamphetamine in the urine sample was 1.36. Based on the two values, the retention times of standard methamphetamine and urine samples are almost the same.

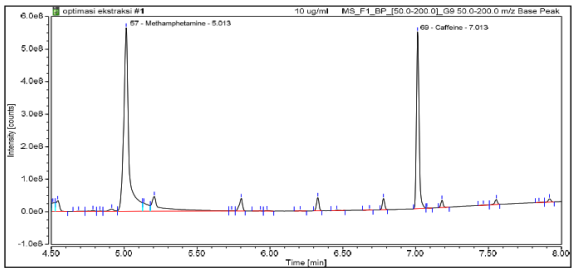

Figure 3. Chromatogram of a standard solution of methamphetamine 10ug/~~ml~~mL in acetonitrile with a value of tR (minutes) = 5.013

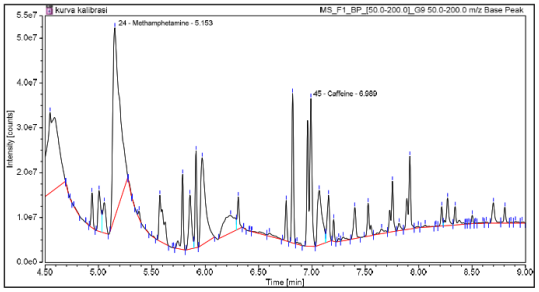

Figure 4. Chromatogram of the methamphetamine solution extracted from urine in acetonitrile solution with a value of tR (minutes) 5.153

The results of ~~the identification of~~identifying the mass spectrum of methamphetamine in urine compared to the mass spectrum of methamphetamine in the NIST database library were in Figure 5. Based on this ~~-~~figure, the essential peak of the analyte in the urine sample is the same as methamphetamine in the literature, namely m/z = 58, and caffeine as an internal standard with an

m/z = 194. Match Factor (SI) or Reverse Match Factor (RSI) values can use as a reference for the quality interpretation of the mass spectrum of the analyte. The match factor data for methamphetamine is 840 (good), and the internal standard is 800 (good).

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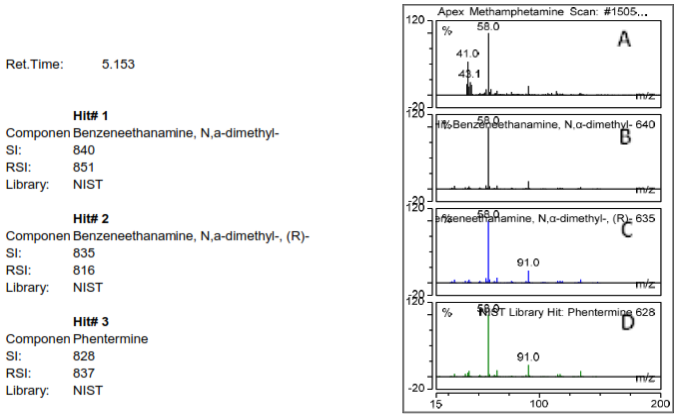

Figure 5. Mass spectrum of methamphetamine versus NIST literature, sample spectrum (A); the spectrum of NIST Hit 1 (B), Hit 2 (C), Hit 3 (D).

3.4. Validation method

The validation method was carried out by assessing several analytical parameters based on the Bioanalytical Method Validation M10 by International Council for Harmonisation (ICH), European Medicines Agency (CHMP, 2019), such as selectivity, specificity, linearity, limit detection, limit quantitation, effect matrix, carry-over, accuracy and precision.

3.4.1. Selectivity

The evaluation of selectivity using samples of methamphetamine containing IS, then the calculation of resolution (Rs) values of methamphetamine and IS against other ~~components that were closest~~closest components. The value of Rs for methamphetamine ~~is~~ was 4.62, and the IS ~~is~~ was 2.07. Based on these resolution values, it concluded that the method is selective because it can separate the methamphetamine peaks from the peaks of other components with a value of Rs > 1.5.

3.4.2. Specificity

The specificity analysis showed the absence of other components that interfere with the retention time of methamphetamine and IS retention time in the blank sample. In addition, specificity can also assess from the relative retention time of methamphetamine to IS in standard

solution, which is 1.36 compared to a retention time relative methamphetamine in a urine sample, 1.39; both retention times are almost the same. Another evaluation of the specificity test is the match factor score (SI) (Gujar et al., 2018). Where the SI score for methamphetamine using this method is 840 (good match), the results of which can be seen in the qualitative test (figure 5). The results from the specificity test show that the method used is specific.

Evaluation of the selectivity and specificity for IS are assessments of the value of Rs for the internal standard is 2.07 > 1.5. There is a disturbing peak at IS retention time, but the percentage of interfering response is 5.8%, which is not more than 20% of the IS response at a concentration of 2.5 µg/~~nl~~mL. Based on that evaluation, the conclusion is the method specific to IS.

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3.4.3. Linearity, Detection Limit, Quantitation Limit, and Effect Matrix.

The results of observing the peak area of methamphetamine from standard solutions for the determination of linearity, limit of detection (LOD), the limit of quantitation (LOQ), and matrix effects can be calculated from the price of the sensitivity of the slope (Sl) based on a comparison between levels and peak area as shown in table 4 and figure 6.

Table 4. The ~~R~~relative area of methamphetamine to IS sample blank, internal standard (IS), standard 1 to standard 6 (STD 1 - STD6), Mean, %CV and %Bias (n=3).

	Blank	**IS**	**STD 1**	**STD 2**	**STD 3**	**STD 4**	**STD 5**	**STD 6**
Concentration (µg/~~nl~~mL)	0	2,5	5	6	7	8	9	10
Replication 1	0,07	0,16	1,28	2,28	3,70	4,60	5,80	6,60
Replication 2	1,24	0,16	1,44	2,55	3,95	4,59	5,87	6,58
Replication 3	0,67	0,19	1,43	2,52	3,49	4,65	5,50	6,56
Mean			1,38	2,45	3,71	4,61	5,72	6,58
%CV			6,48	6,04	6,20	0,70	3,43	0,30
%Bias			1,39	0,83	2,17	0,12	0,76	1,12

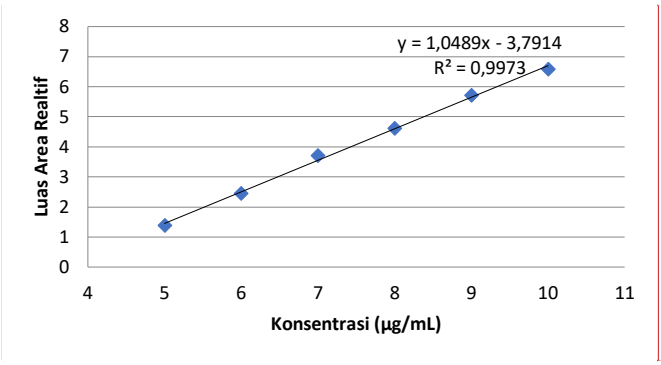

Figure 6. Methamphetamine calibration curve for determination of linearity, LOD and LOQ

The correlation test results between variable x (concentration) and variable y (relative area) using SPSS 21.0, where the significance value = 0.000 is smaller than the value α = 0.05, so there is a correlation or a relationship between concentration and relative area. The Pearson correlation (r) = 0.996 shows a positive relationship with the degree of perfect correlation between concentration and relative area (Samuels, 2015). The higher the concentration, the higher the relative area value. Table 4 shows the matrix effect of all concentration levels in the linearity range: an average accuracy value is 99.9%, while for precision, the average %CV value is 3.86%. From these results, the components in the sample matrix do not disturb the analysis process.

3.4.4. Accuracy and Precision (Intraday and Interday)

Determine the accuracy (% recovery) and precision (% coefficient of variation or %CV) through three concentration levels of methamphetamine were added to the urine, namely 5µg/~~ml~~mL as a low concentration, 7µg/~~ml~~mL as a middle concentration, and 9µg/~~ml~~mL as a high concentration. The results of the accuracy and precision tests in Table 5 are both carried out intraday (same day) and interday (three different days).

Table 5. Accuracy (%R ± SD) and precision (%CV) test results intraday and interday (n=3).

True Value (µg/~~ml~~mL)	*Intraday*			*Interday*		
	Mean Concentration (µg/~~ml~~mL)	%CV	%R ± SD	Mean Concentration (µg/~~ml~~mL)	%CV	%R ± SD
5	4,~~-~~93	1,~~-~~77	98,~~-~~7 ± 1,~~-~~75	4,~~-~~98	4,~~-~~85	99,~~-~~6 ± 4,~~-~~83
7	7,~~-~~15	3,~~-~~06	102 ± 3,~~-~~14	5,~~-~~95	3,~~-~~89	85,~~-~~0 ± 3,~~-~~32

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9	9,07	2,08	101 ± 2,10	8,57	14,0	95,2 ± 13,5

Table 5 shows that the %R intraday values at three concentrations were 98.7 ± 1.75% at low concentrations, 102 ± 3.14% at middle concentrations, and 101 ± 2.10% at high concentrations. The %R interday at three concentrations were 99.6 ± 4.83% at low concentration, 85.0 ± 3.32% at middle concentration, and 95.2 ± 13.5% at high concentration. The precision test at three-level concentrations (%CV) for intraday analysis was 1.77% at a low level, 3.06% at the middle, and 2.08% at a high level. The %CV interval at three concentrations was 4.98% at a low concentration, 5.95% at a medium concentration, and 8.57% at a high concentration.

From the overall accuracy and precision test results both intraday and interday, the accuracy (%R) is between 85-115%, and the precision test (%CV) is <15%, so the m-QuEChERS method used has high accuracy and precision (Riyanto, 2002).

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3.4.5. Stability Test

Injections at one concentration level were stored for 2 hours to 8 hours at room temperature and replicated three times. The results of the stability test are in Table 6.

Table 6. Stability test results, %CV and % reduction in methamphetamine concentration during storage time of 0, 2, 4 and 8 hours (n=3).

Time (hour)	Mean Concentration (µg/~~ml~~mL)	SD	%CV	% Reduction
0	4,38	0,056	1,27	-
2	4,30	0,010	0,24	1,95
4	4,24	0,041	0,97	3,35
8	4,05	0,240	5,90	7,80

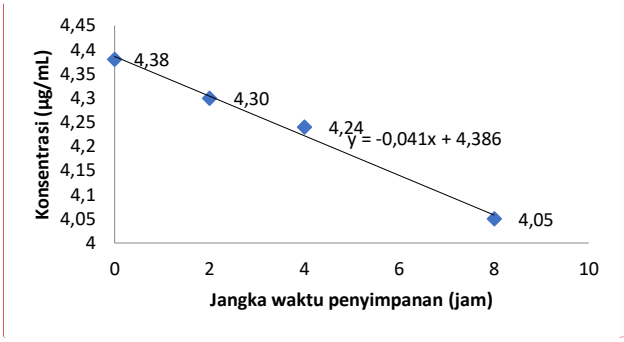

Figure 7. Methamphetamine concentration reduction curve during storage at room temperature 30°C at storage times of 0 hours, 2 hours, 4 hours, and 8 hours.

From the stability test, there was a decrease in the concentration of methamphetamine by 1.95% at 2 hours of storage, 3.35% at 4 hours, and 7.80% at 8 hours of storage. From the linear equation data on the graph, the stability of determining methamphetamine levels decreased by 0.041 µg/~~ml~~mL-mL per hour. This stability test result is a basis for analysis that urine samples must be processed immediately upon receipt because the concentration of methamphetamine will continue to decrease during storage at room temperature 30°C so that the measured concentration will be lower than the actual concentration.

3.4.6. Carry Over test

During the validation process, the assessment of the carry-over parameter by analyzing the blank sample after the highest calibration standard for the analyte and internal standard to see changes in the measured concentration due to residual analyte from the previous sample remaining in the analytical instrument. Following the highest standards, the carry-over in the blank sample should not be more significant than 20% of the methamphetamine response at 3 µg/~~ml~~mL and 5% of the IS response at 2.5 µg/~~ml~~mL. ~~The~~ T~~t~~able 7 shows the results of the carry-over test.

Table 7. The area of methamphetamine in the blank sample followed the highest standard (10 µg/~~ml~~mL) and the percentage of the area of methamphetamine at a concentration of 3 µg/~~ml~~mL and to IS at a concentration of 2.5 µg/~~ml~~mL.

Methamphetamine			Interal Standard (IS)		
Area Analyte	Area analyte at 3µg/~~ml~~mL	% Respon	Area Analyte	Area IS at 2.5µg/~~ml~~mL	% Respon
45.876	996.692	4.~~,~~60	37051	1.519.376	2.~~,~~44
24.074	996.692	2.~~,~~42	36198	1.519.376	2.~~,~~38

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162.057	996.693	16,3	44963	1.519.376	2,96
Average of analyte response		7,76	Average of IS response		2,59

From Table 7, the percentage area of methamphetamine in the blank sample following the 10ug/~~ml~~mL standard was an average of 7.76% of the peak area of methamphetamine at 3µg/~~ml~~mL and 2.59% the peak area of IS at 2.5µg/~~ml~~mL. These results indicated that minimalization of carry-over during the analysis process succeeded because the average percentage of methamphetamine and IS responses is less than 20%.

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3.4.7. Application of m-QuEChERS method for methamphetamine determination in the urine of abusers.

Determination of urine samples from 4 suspected methamphetamine abusers using a rapid test (immunoassay) method. The urine was extracted using the selected m-QuEChERS method and injected into gas chromatography-mass spectroscopy. ~~The t~~Table 8 shows the results of methamphetamine determination in the urine of the abuser.

Table 8. Analysis results of methamphetamine in the urine of four patients who abuse methamphetamine using the rapid test method (immunoassay) and gas chromatography-mass spectroscopy

Abuser	Rapid Test Result	Mean Concentration of Metamfetamin (µg/~~ml~~mLmL) duplo	SD	%CV
P1	~~positif~~positive	25,51	1,70	6,66
P2	positive~~positif~~	15,05	0,95	6,31
P3	positive~~positif~~	17,72	0,39	2,21
P4	~~negatif~~negative	3,08	0,10	3,38

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Based on Tabel 8, the results of 4 urine samples of methamphetamine abusers, three samples (P1, P2, and P3) gave consistent results between the rapid test and the confirmatory test using gas chromatography-mass spectroscopy, but sample 4 (P4) could not detect methamphetamine levels which was~~is~~ too low so that there ~~is~~ was a difference in results between the rapid test and the gas chromatography-mass spectroscopy test. The inability of the rapid test to detect methamphetamine may be due to abusers having used methamphetamine beyond their detection limit of 3-4 days. However, gas chromatography tests can still detect methamphetamine at 3.08 µg/~~m~~mL~~l~~ levels.

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4. Conclusion

A rapid, selective, ~~spesifi~~specific~~c~~, and reliable method for ~~the analysis of~~analyzing methamphetamine in urine was developed. The validation results of the m-QuEChERS extraction method met the validation criteria according to the standard validation method, namely the ICH guidelines Bioanalytical Method Validation M10 on all aspects of the validation tested, namely selectivity and specificity, matrix effect, linearity, accuracy, precision, and carry over. The m-QuEChERS method can be applied to routine laboratory testing to analyze methamphetamine in the urine of methamphetamine abusers.

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Appendix A. Suplplementary Data

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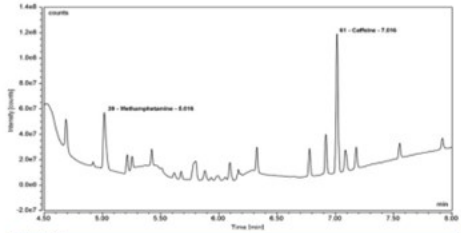

Peak Results						
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