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Research Paper

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

Soraya Aulia[1,2], Riesta Primaharinastiti[1], Djoko Agus Purwanto[1*]

[1]*Magister of Pharmaceutical Science, Airlangga University, Nanizar Zaman Joenoes Building, Campus C, Mulyorejo, Surabaya, 60115, Indonesia*
[2]*Laboratory of Chemistry and Toxicology, Laboratory Center of Health, Testing and Calibration, West Nusa Tenggara Provincial Health Office, Caturwarga Street Number 4, Mataram, 83121, Indonesia*
*__Corresponding author__: djokoagus@ff.unair.ac.id

Abstract
This paper explains the development of a quick and easy gas chromatography (GC) approach to identify methamphetamine in urine. This research used gas chromatography with mass spectroscopy and a capillary column TG-5SILMS (5% phenyl methyl siloxane, 30 m x 0.32 x 25 m). The carrier gas flow rate was set at 1.0 mL/minute, the temperature inlet and detector had been set at 300°C, and the oven temperature was programmed to initiate at 50°C and held for 1.5 minutes before being raised to 300°C at a rate 40°C/minute 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. The optimal conditions for processing a 400 μL urine sample were 160 mg magnesium sulphate, 40 mg sodium chloride, and 400 μL acetonitrile for organic solvent. According to the validation test, the detection limit for methamphetamine was 0.36 μg/mL; the quantitation limit was 1.09 μg/mL, and the calibration curve followed 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 9 μg/mL on average was 100.5±2.33% for intraday dan 93.3±7.21% for interday. The precision was excellent, with an average coefficient of variation of 2.31%. The procedure was applied to four urine samples from drug users and the first abuser (25.51 μg/mL), the second abuser (15.05 μg/mL), the third abuser (17.72 μg/mL) and the last abuser (3.08 μg/mL) were all satisfactorily quantitated.

Keywords
GC-MS, QuEChERS, Urine, Methamphetamine, Validation

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1. INTRODUCTION

As a world body dealing with narcotics issues, the United Nations Office on Drugs and Crime (UNODC) notes that at least 271 million people worldwide, or 5.5% of the total global population aged 15 to 64 years, have consumed drugs, with at least one person having 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 (National, 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., 2019).

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 an amphetamine that has a methyl substituent in the amino group (S)-amphetamine. It is a neurotoxin, a psychotropic medication, a central nervous system stimulant, a xenobiotic, and an environmental pollutant (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 and 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 (Volkow et al., 2010).

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. Analyte extraction and purification are critical in detecting medicines and metabolites in biological materials. Traditional sample extraction or purification methods such as liquid-liquid extraction (LLE) or solid phase extraction (SPE) consume 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; Maggira and Samanidou, 2018; Stevens and Jones, 2010; Westland and 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 (Carasek et al., 2022; Lawal et al., 2018; Seidi et al., 2019).

Anastassiades et al. (2003) developed the QuEChERS (rapid, easy, cheap, effective, rugged, and safe) approach for evaluating 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 (Numbers, 2017). The QuEChERS method is similar to LLE but highly selective, like SPE. The QuEChERS method involves extracting the sample with acetonitrile or ethyl acetate solvents and dehydrating it in salts such as magnesium sulphate and sodium chloride (Asl 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 sulphate 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 (Numbers, 2017). Asl et al. (2017), carried out another study. They used 400mg of magnesium sulphate and 200mg of sodium chloride with a volume of 1 mL of the urine sample and added buffer until the sample pH became 8-9. This study produced a fairly good recovery value of 78% (Asl et al., 2017).

Numerous investigations have been conducted to modify and optimize the QuEChERS method, including optimizing the use of solvents and partition salts based on the target analyte to be analyzed, as well as modifications to 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 and Snow, 2016).

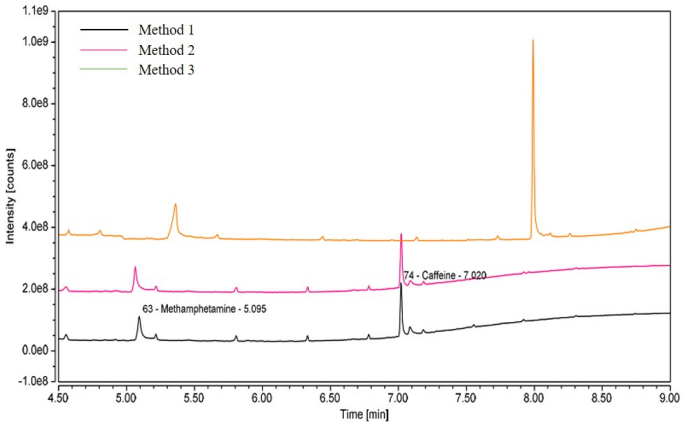

Figure 1. Overlay 10 μg/mL Methamphetamine Chromatogram Method 1, 2, and 3 in Acetonitrile Using Gas Chromatography.

The QuEChERS modification is also very important, especially when the number of samples available is limited, and is very popular in analytical chemistry because of its advantages such as lower solvent, salt, and sorbent costs, simpler handling, processing, and elimination of waste when compared to traditional extraction procedures. This feature provides higher throughput analysis, resulting in increased accuracy and significant savings in time and expense. The development of the QuEChERS technique pushes the issue of miniaturization and automation even further (Perestrelo et al., 2019). Several studies in the forensic field have been carried out to modify QuEChERS through miniaturization of QuEChERS, but the derivatization and evaporation stages are still being carried out (Amorim Alves et al., 2017;Matsuta et al., 2013; Pouliopoulos et al., 2018).

Based on the description above, it is necessary to conduct research related to modifying QuEChERS through miniaturization of QuEChERS (m-QuEChERS) to extract methamphetamine in urine before being analyzed using gas chromatogr aphy-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, $MgSO_4$, NaCl and potassium carbonate were purchased from Merck Indonesia. The standard methamphetamine hydrochloride 1000 μg/mL in methanol was purchased from Cayman Chemical. The Internal standard (caffeine solution 1000 μg/mL in methanol) was purchased from Supelco (USA); The remaining reagents and solvents used were

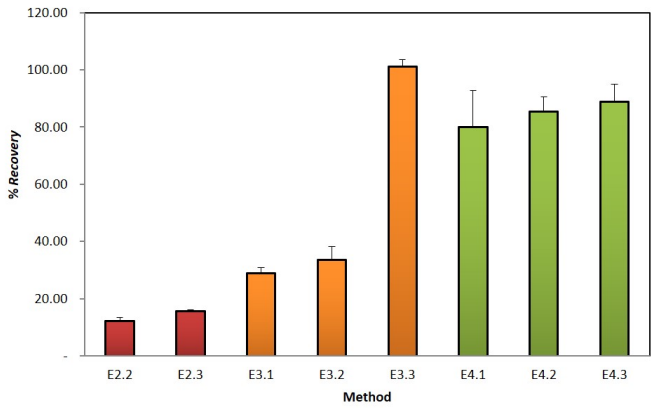

Figure 2. Methamphetamine Recovery Diagram by the m-QuEChERS Method. Red, Extraction Volume 400 μL with Extraction Cycles 2 Times (E2.2) and 3 Times (E2.3). Orange, Extraction Volume 800 μL, Extraction Cycle 1 Time (E3.1), 2 Times (E3.2) and 3 Times (E3.3). Green, Extraction Volume 1200 μL, Extraction Cycle 1 Time (E4.1), 2 Times (E4.2) and 3 Times (E4.3)

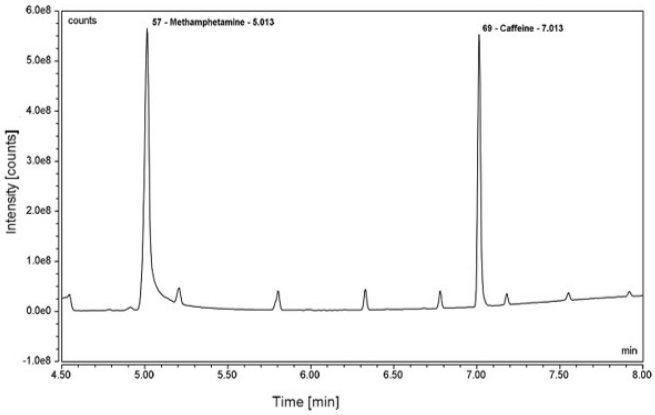

Figure 3. Chromatogram of a Standard Solution of Methamphetamine 10μg/mL in Acetonitrile with a Value of tR (Minutes) = 5.013 and 7.013 for IS (Caffeine)

of analytical grade or above. Water that has been deionized and distilled (Millipore System). Disposable 2 mL safe-lock test tube and 15ml tubes with screw cap (Eppendorf, Germany).

2.1.2 Urine Samples

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

2.2 Methods

2.2.1 Sample Preparation Method

Magnesium sulphate and NaCl were crushed and thoroughly combined in a weight ratio of 4:1. The mixture was weighed into disposable 2 mL safe lock test tubes (200 mg each, 5 mg). A 400 μL of acetonitrile was poured into disposable 2 mL safe lock test tubes holding the $MgSO_4$ and NaCl combination. A 400 μL urine sample was adjusted to a pH of more than 10 using K_2CO_3 buffer and placed in the test tube. Immediately vortex-mixed for 1 minute, then centrifuged at 10.000 RPM for 5 minutes using a high-speed refrigerated centrifuge MPW-150 (Med. Instrument, Polandia). A pipette was used to separate the organic phase. To recover the organic extract contained in the cake of an inorganic salt, 400 μL of acetonitrile was added to the test tube, and the organic phase was sampled after vortexing and decanting twice. These organic extracts were mixed and evaporated in a gentle nitrogen stream. The recovered residue was dissolved in 400 μL of acetonitrile. (Correia-Sá et al., 2018; Numbers, 2017; Asl et al., 2017; Matsuta et al., 2013; Schmidt and Snow, 2016; Westland and Dorman, 2013).

2.2.2 GC-MS

Sample injection was done manually with a volume of 1 μL. 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/minute 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/minute 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 were set at 300°C. The MS ionization system used Electron Impact (EI) with a strength of 70eV at 300°C. The Thermo Scientific Chromeleon Chromatography Data System (CDS) software is used for data processing and operational systems.

3. RESULTS AND DISCUSSION

3.1 Optimization of GC-MS Conditions

The conditions were optimized by adjusting the rate of increase in oven temperature, gas flow rate, injection mode and sample volume. In this research, there are three different optimization methods. Method 1 uses a sample volume and flow rate of 1 μl with a temperature increase of 40°C. In methods 2 and 3, the sample volume is 2 μl with a flow rate of 1.5 μl, but the speed of temperature rise is different, namely 40°C for method 2 and 30°C for method 3.

From Table 1 and Figure 1, the retention time (tR) of methamphetamine was 5.095 minutes, and IS was 7.020 for method 1. Method 2 showed methamphetamine's tR was 5.064 minutes and IS 7.023, while method 3 showed methamphetamine's tR was 5.390 minutes and IS 7.993. The results of gas chromatography optimization were assessed from several parameters, namely resolution, theoretical plate number and match factor, which can be seen in Table 1 (Granquist et al.,

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/min)	Temperature Rising Speed	Injection Mode	Sample Volume (μL)	Relative Retention Time met/IS (min)	Resolution met/IS*	Theoritical Plate Numbersmet./IS	Relative Area met/IS*	MF* 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

*MF = Match Factor; Met = Methamphetamine; IS = Internal Standard (Caffeine)

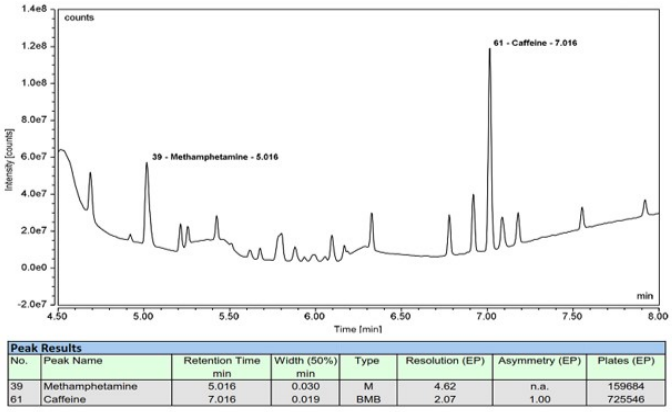

Figure 4. Chromatogram of the Methamphetamine Solution Extracted from Urine in Acetonitrile Solution with a Value of Retention Time/tR (Minutes) 5.016 and Resolution (Rs) 4.62. For IS (Caffeine), tR (Minutes) 7.016 and Resolution (Rs) 2.07

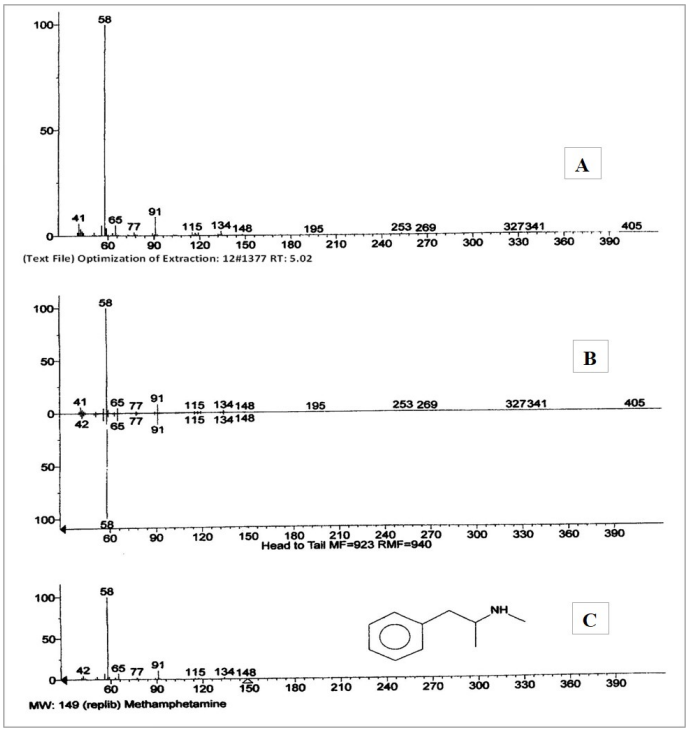

Figure 5. Mass Spectrum of Methamphetamine in Urine (A) with RT = 5.02; Mass Spectrum Methamphetamine in Urine Versus NIST Library Database (B) with MF = 923 and Mass Spectrum Methamphetamine NIST Library Database (C) with MW = 149

2019).

The result of optimizing the selected gas chromatography conditions was method 1: an initial temperature of 50°C for 1 minute with a temperature increase of 40°C/minute until it reaches 300°C, which is maintained for 3 minutes. The inlet temperature was 300°C, the splitless mode, and the gas flow rate was 1.0 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 were less than methods 2 and 3, namely 1 mL/minute, 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. Methods 1, 2 and 3 show a resolution value of 3.85, 5.08 and 3.53, where the three values meet the requirements for good analyte resolution, which is greater than 1.5. Likewise, with the theoretical plate number, which showed a value greater than 10,000, the column can separate the analyte from the mixture. The match factor (MF) value showed the similarity of the analyte spectrum with the reference spectrum in the library, which can be seen in Table 1; the three methods showed an MF value of > 800, which means that the level of similarity is good according to the NIST library guidelines (Gujar et al., 2018).

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 $MgSO_4$ 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 done by modifying several extraction parameters: the number of inorganic salts, extraction volume and extraction cycle. Differences in variation can be seen in Table 2.

The results of standard extraction of 10 μg/mL metham-

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 (µL)	Acetonitrile Volume (µL)	Extraction Volume (µL)	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)

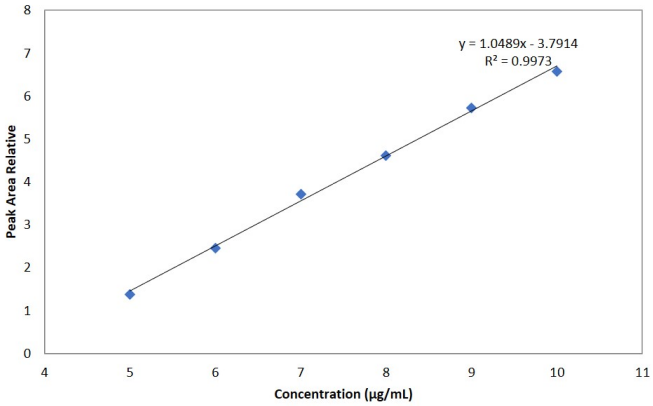

Figure 6. Methamphetamine Calibration Curve for Determination of Linearity, LOD and LOQ

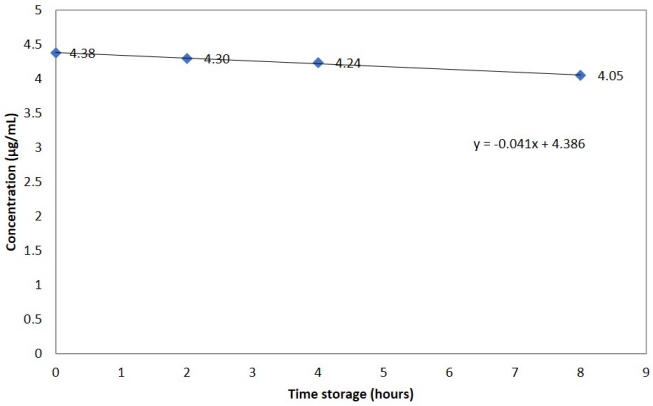

Figure 7. Methamphetamine Concentration Reduction Curve During Storage at Room Temperature 30°C at Storage Times of 0, 2, 4, and 8 Hours

phetamine in urine with several variations in extraction volume can be seen in Table 3, where the relative area was obtained from the ratio of the methamphetamine area to the IS area, then %CV and %Recovery (%R) was calculated (Campêlo et al., 2021). The recovery percentage 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). Table 3 shows 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. After all, the extraction volume was very small, namely 100 μL, so the analysis results were not obtained. Extraction method 2 with one 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 (Matsuta et al., 2013).

It can be seen in Figure 2 that the extraction that gives the best % recovery results is the E3 extraction volume (800 μL) for three extraction cycles with a %R of 101.2 ± 2.30% and a %CV of 2.27%. According to Orfanidis et al. (2022), recoveries of the analyte were satisfactory was more than 85%. The next extraction stage was performed using these conditions (Orfanidis et al., 2022).

3.3 Qualitative Test of Methamphetamine in Urine Samples by GC-MS

The two chromatogram images (Figure 3 and Figure 4) showed that the standard methamphetamine retention time is 5.013 minutes, and in the urine sample is 5.016 minutes. The relative retention time for the solution and the methamphetamine in the urine sample was 0.71. The ratio of methamphetamine

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 (times)	Visual	Mean Relative Area met./IS	Recovery ± SD (%)	%CV
E1	1	The Organic Phase	n.a	n.a	n.a
	2	Layers are Difficult to	n.a	n.a	n.a
	3	Separate	n.a	n.a	n.a
E2	1	Cloudy Organic Phase	n.a	n.a	n.a
	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

n.a = not available = no analysis was performed by GC-MS

Table 4. The 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/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

retention time to IS (caffeine) retention time yields the relative retention time. Based on the two values, the relative retention times of standard methamphetamine and urine samples are the same.

The results of identifying the mass spectrum of methamphetamine in urine compared to the mass spectrum of methamphetamine in the NIST library database are in Figure 5. The best compound identification is generally assessed from the highest match factor (MF), which represents the suitability between the measured compound spectrum and the reference spectrum. For this reason, this study used the NIST library guidelines to interpret the MF values of the analytes tested as a reference for interpreting the quality of the mass spectra of analytes.

Based on the standard chromatogram of methamphetamine in urine samples, the five highest m/z values were for methamphetamine, namely 58, 91, 56, 65 and 42. Figure 5 shows that the MF value for methamphetamine was 923 (excellent match). An excellent match means that the analyte (methamphetamine) was identical to the NIST library database (Gujar et al., 2018).

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 (Guideline, 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 closest components. The value of Rs for methamphetamine was 4.62, and the 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 (Woźniak et al., 2018).

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 ad-

Table 5. Accuracy (%R ± SD) and Precision (%CV) Test Results Intraday and Interday (n=3)

True Value (µg/mL)	Intraday			Interday		
	Mean Concentration (µg/mL)	%CV	%R ± SD	Mean Concentration (µg/mL)	%CV	%R ± SD
5.0	4.93	1.77	98.7 ± 1.75	4.98	4.85	99.6 ± 4.83
7.0	7.15	3.06	102 ± 3.14	5.95	3.89	85.0 ± 3.32
9.0	9.07	2.08	101 ± 2.10	8.57	14.0	95.2 ± 13.5

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)	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

dition, specificity can also assess the relative retention time of methamphetamine to IS in standard solution compared to a retention time relative to methamphetamine in a urine sample; both retention times are the same, namely 0.71. Another evaluation of the specificity test is the match factor score (MF) (Gujar et al., 2018). Where the MF score for methamphetamine using this method is 923 (excellent 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 (Numbers, 2017). 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/mL. Based on that evaluation, the conclusion is the method specific to IS (Guideline, 2019).

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

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. The linearity of the method was studied within the range of the mean therapeutic concentration of methamphetamine, which was found in the literature (Kim et al., 2004; Volkow et al., 2010). Every standard was analyzed in three replicates of the IS. 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). If the analyte concentration is high, the relative peak area of the analyte will also be higher. From Table 4 and Figure 6, it can be calculated that the price S(y/x) = 0.11443 and the slope value (Sl) = 1.0489. LOD is calculated from 3.3S(y/x)/Slope and LOQ from 10S(y/x)/Slope. Therefore the detection limit value obtained is LOD = 0.36 μg/mL and the quantity limit is LOQ = 1.09 μg/mL. 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%. The accuracy should be around 15% of the nominal concentration, and the precision (per cent coefficient of variation (%CV)) should not be more than 15% in all individual matrix sources/lots, according to ICH guideline M10 on bioanalytical method validation. These results met its requirement, meaning components in the sample matrix do not disturb the analysis process (Guideline, 2019).

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 5.0 μg/mL as a low concentration, 7.0 μg/mL as a middle concentration, and 9.0 μg/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 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

Table 7. The Area of Methamphetamine in the Blank Sample Followed the Highest Standard (10 µg/mL) and the Percentage of the Area of Methamphetamine at a Concentration of 3 µg/mL and to IS at a Concentration of 2.5 µg/mL

Methamphetamine			Internal Standard (IS)		
Area Analyte	Area Analyte at 3 µg/mL	% Respon	Area Analyte	Area IS at 2.5 µg/mL	% Respon
45876	996692	4.60	37051	1519376	2.44
24074	996692	2.42	36198	1519376	2.38
162057	996693	16.3	44963	1519376	2.96
Average of Analyte Response		7.76	Average of IS Response		2.59

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 Methamphetamine (µg/mL) Duplicate	SD	%CV
P1	Positive	25.51	1.70	6.66
P2	Positive	15.05	0.95	6.31
P3	Positive	17.72	0.39	2.21
P4	Negative	3.08	0.10	3.38

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 (Guideline, 2019;Kaur and Sharma, 2018).

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. 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 per hour (Figure 7). 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 (Guideline, 2019; Pouliopoulos et al., 2018).

3.4.6 Carry Over Test

Throughout the validation procedure, the carry-over parameter is evaluated by examining the blank sample following the highest calibration standard for the analyte and internal standard to check for any variations in the measured concentration due to residual analyte from the prior sample remaining in the analytical equipment. 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 and 5% of the IS response at 2.5 μg/mL. Table 7 shows the results of the carry-over test. The percentage area of methamphetamine in the blank sample following the 10μg/mL standard was an average of 7.76% of the peak area of methamphetamine at 3 μg/mL and 2.59% of the peak area of IS at 2.5 μg/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% (Guideline, 2019).

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 spectrosco py. Table 8 shows the results of methamphetamine determination in the urine of the abuser. 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 were too low so that there 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/mL levels (Schmidt and Snow, 2016).

4. CONCLUSION

A rapid, selective, specific, and reliable method for 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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