DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD OF 3, 4-METHYLENEDIOXY-N-ETHYLAMPHETAMINE IN DRIED BLOOD SPOT USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

Objective: This study aims to develop and validate the analytical method to determine 3,4-Methylenedioxy-N-ethylamphetamine (MDEA) in DBS using GC-MS.

Methods: This research used liquid-liquid micro-extraction for sample preparation and analysis was performed by GC-MS. In the method development, the optimized parameters were flow rate, column temperature, the spot of blood volume, % haematocrit, extraction and reconstitution of solvent volume, and sonication duration. Validation of the chosen method was performed based on EMEA bioanalytical guideline in 2011.

Results: The optimum chromatographic conditions were obtained using HP-5 MS capillary columns (30 m x 0.25 mm i.d; 0.25 μm); helium with 99.9% purity as a mobile phase; flow rate of 1.0 ml/min; column temperature was 250 °C. MS detection using 4 fragments at m/z values of 72.00 and 44.00 for MDEA and 58.00 and 77.00 for ephedrine HCl as an internal standard. The DBS paper with the volume of blood spot 40 μl was then extracted using liquid-liquid micro-extraction with methanol 700 μl, sonication for 5 min, evaporated with nitrogen gas then reconstituted with 50 μl ethyl acetate. The validation results fulfilled the requirements based on the EMEA bioanalytical guideline in 2011.

Conclusion: It can be concluded that the optimum condition of the analytical method by using GC-MS was obtained and fulfilled validation criteria with a range concentration of 15-250 ng/ml.

Keywords: DBS, Ephedrine HCl, GC-MS, Liquid-liquid microextraction, MDEA, Validation

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INTRODUCTION

Drug abuse has been increasing in recent years. Based on World Drug Report on 2017, it is said that the prevalence of drug abuse is 4.8% in 2009, increased to 5.2% by 2011, and to 5.3% by 2015 [1]. One of the abused substances was amphetamine type-stimulant (ATS), such as 3,4-Methylenedioxy-N-ethylamphetamine (MDEA) [2]. Amphetamine is part of the psychotropic groups as one of the Narcotics, Psychotropic, and Addictive Substances. According to Indonesia Law No. 5 y 1997, psychotropic substances or drugs are either natural or synthetic that can lead to psychoactive efficacy in the form of selective influence on the central nervous system that causes a distinctive change in mental activity and behavior. Psychotropic can be classified into four groups: Group I, Group II, Class III, and Group IV. However, since the enactment of Indonesia Law no. 35 y 2009, psychotropic class I and class II have been classified as narcotics [3].

MDEA belongs to class I drug according to the regulation [3]. This compound is an entactogen and belongs to the amphetamine in Indonesia type and the class of phenylethylamine [4]. To declare status of a person is positive using drugs, it must be proven through the identification of the abused compounds in biological matrices such as blood, urine, hair, and saliva. There are some drawbacks in the use of the matrix. In the urine sample, it is susceptible to counterfeiting and sometimes is considered as a privacy violation. In the hair samples, the deficiencies are the expensive and the use of recently used drugs cannot be detected. In saliva samples, the deficiency is in the form of drug availability is only slightly in time compared to urine [5]. In the narcotic drug analysis, tests performed using the new method, dried blood spots (DBS) are still few in numbers. Thus, it is necessary to do research using DBS [6]. This DBS method uses filter paper and blood samples. The advantage of this method, is that it only needs small volume of blood, not invasive to the body, stable sample, and easy to handle [7].

The first step of analysis is sample preparations, namely protein precipitation method, solid-phase extraction and liquid-liquid extraction. Based on existing methods, there are some shortfall. Protein precipitation methods can produce large amounts of impurities. The solid phase extraction method requires a longer step than liquid-liquid extraction process and can sometimes cause irreversible adsorption of a number of analyte on the used cartridge [8]. Liquid-liquid extraction has a deficiency in terms of solvent toxicity [9] and large amounts of solvents with high polarity and often emissions [10]. One of the recent developed sample preparation method is micro-extraction by simplifying sample preparation procedures and improving the quality and sensitivity of the analysis [11, 12]. In addition, the advantages are fewer solvents and less time needed7. The technique can be applied to the analysis of drug abuse on a small sample with short analysis time.

For analysis, there are two instruments that are commonly used, Liquid Chromatography (LC) and Gas Chromatography (GC), both with mass spectrometry (MS). GC-MS provided some advantages such as lower cost and the analysis process can be faster. GC-MS can be used to especially analyze compounds that are volatile and heat-resistant [13]. Therefore, this study aimed to develop and validate MDEA analytical method in DBS using GC-MS. Hopefully, a method of MDEA analysis in DBS that is validate can be developed, thus can be widely applied.

MATERIALS AND METHODS

Materials–

The instruments used in this study were Gas Chromatography-Mass Spectrometry (Shimadzu GC-MS QP2010 Ultra), equipped with HP-5 MS Capillary Column (30 m x 0.25 mm; 0.25 μm ), a syringe (Shimadzu), Helium Gas with purity 99.99%, DBS paper (Perkin Elmer), micropipette (syracex acura) and tip, vial GC (Agilent), glass tube inserted on GC vial (Agilent), sonicator, and other glass tools such as beaker glass and tube (Iwaki), MDEA 1000 ppm in liquid form, 1 ml ampoule (Cerilliant), Ephedrine HCl (BPFI) as internal standard. The optimum parameters were flow rate, column temperature, spot of blood volume, % haematocrit, extraction and reconstitution of solvent volume, and sonication duration. Validation of the chosen method was performed based on EMEA bioanalytical guideline in 2011.

The optimum chromatographic conditions were obtained using HP-5 MS capillary columns (30 m x 0.25 mm i.d; 0.25 μm); helium with 99.9% purity as a mobile phase; flow rate of 1.0 ml/min; column temperature was 250 °C. MS detection using 4 fragments at m/z values of 72.00 and 44.00 for MDEA and 58.00 and 77.00 for ephedrine HCl as an internal standard. The DBS paper with the volume of blood spot 40 μl was then extracted using liquid-liquid micro-extraction with methanol 700 μl, sonication for 5 min, evaporated with nitrogen gas then reconstituted with 50 μl ethyl acetate. The validation results fulfilled the requirements based on the EMEA bioanalytical guideline in 2011.

Conclusion: It can be concluded that the optimum condition of the analytical method by using GC-MS was obtained and fulfilled validation criteria with a range concentration of 15-250 ng/ml.
standard, Methanol (Merck), HCl (Merck), Ethyl Acetate (Merck) and Blood (Indonesian Red Cross).

Method validation results have been conducted in the form of LLOQ measurements, linearity calibration curve, selectivity, accuracy, precision, carryover, matrix effect, and stability. All parameters met the requirements based on EMEA bioanalytical guidelines in 2011.

In this research, optimization was conducted on some variables such as flow rate, column oven temperature of GC-MS, amount of MS fragments, blood spot volume, % hematocrit, extraction and reconstitution solvent volume, and sonication duration. When optimized flow rate, column temperature, and amount of MS fragments, MDEA standard solution and Ephedrine HCl 1 ppm (1000 ng/ml) was injected into GC-MS. The variation used for flow rate optimization were 0.8; 1.0; 1.2 ml/min with column temperature set into 250°C at 10°C/min. The results were compared and the chosen condition was assessed based on peak retention time and size of the analytical area. The same thing was done when optimized column temperature by using the chosen flow rate from the previous optimization. Variation are 250; 280; 300 °C, each at 10 °C/min. MS optimization was performed by first comparing full scan mode at range m/z 50-550 and selected ion monitoring (SIM) mode. After that, optimization was continued by a varying number of fragments. The chosen fragments were based on the high relative intensity of the peak. The chosen fragments for optimization were 4 fragment consisting of 2 MDEA fragments (m/z 72 and m/z 44) and 2 Ephedrine HCl fragments (m/z 58 and m/z 77), 3 fragments are consisting of 1 MDEA fragment (m/z 72) and 2 Ephedrine HCl fragments (m/z 58 and m/z 77), and 2 fragments consisting of 1 MDEA fragment (m/z 72) and 1 Ephedrine HCl fragment (m/z 58).

Afterward a system suitability test was performed using MDEA and Ephedrine standard solution at concentration 1000 ng/ml injected 1 μl into GC-MS by using selected flow rate, selected number of fragments, and selected column temperature. The injection is done five replicas, then observe coefficient of variation to the ratio of peak area as well as the retention time of analyte and internal standard.

In the optimization of DBS sample preparation was done by optimizing the volume of the blood spot, hematocrit, the volume of the reconstitution solution, the volume of the extracting solution, and the time of sonication. When optimized, the volume of blood spot spiked blood sample with a final concentration of 1000 ng/ml of 10, 20, 30, and 40 μl on Perkin Elmer DBS Paper. Then the drying and extraction process was performed by cutting the blood spot on the paper and adding 500 μl methanol and 100 μl internal standard (1000 ng/ml). Afterward, it was sonicated for 15 min using a sonicator. Then prepared a vial containing 10 μl of 0.25% HCl solution in methanol and transferred the extraction results into the vial, dried using nitrogen gas, and reconstituted using 100 μl of ethyl acetate solution and transferring the liquid into a glass insert. After that, 1 μl of the liquid was injected into gas chromatography-mass spectrometry. The selected one will be based on the size of area. In the optimization of % hematocrit, there are 6 variations of hematocrit used, which were 33, 36, 38, 40, 43, and 47%. In this optimization process, spike the blood sample as the selected spot volume of the previous optimization result with the final concentration of 100 ng/ml and the same extraction preparation like before was performed. The parameter needed to be observed in this optimization was the analyte area. The optimization of the volume of the reconstitution solution was carried out by the same extraction as the variations of the 50 and 100 μl reconstitution volumes by using the selected spot volume and % hematocrit from previous optimization results. On the optimization process, the volume of extracting solution was carried out by variation of extracting solution in the form of 300, 500, and 700 μl methanol using the selected reconstitution volume optimization result. The optimization of sonication duration was carried out with variations of 5, 15, and 30 min using the volume of reconstitution and extraction solvent from selected optimization results previously conducted.

After obtaining gas chromatography-mass spectrometry conditions and optimization, the results of DBS preparation were validated according to EMEA bioanalytical guidelines in 2011. Validation parameters performed were the lower limit of quantification measurement (LLOQ), calibration curve, accuracy, precision, selectivity, carryover matrix effect, and stability of the stock solution and DBS samples [14].

RESULTS AND DISCUSSION

The analysis was carried out using GC-MS equipped with HP-5 MS Capillary Column (30 m x 0.25 m m; 0.25 μm ), a syringe (Shimadzu), Helium Gas with purity 99.99%.

Selection of IS

The chosen internal standard must be similar in physicochemical properties with the standard compound. Ephedrine HCl was chosen as an internal standard because it has similar physicochemical properties with MDEA also, both can be separated by using GC-MS. The existence of an internal standard has the function to control the error that may occur in analysis, especially when using a very small concentration in the biological matrix.
Optimization of flow rate and column temperature

Three types of flow rates used were 0.8; 1.0; and 1.2 ml/min. The chosen flow rate and column temperatures are 1.0 ml/min and 250 °C because it has the highest area. The higher flow rate will make retention time become shorter. If the flow rate is lower, it will possibly make the separation is not going well. If the flow rate is too high, it will possibly make column pressure higher, which also can damage the column.

Optimization of the amount of fragments

Analysis of MDEA and ephedrine HCl was done using positive ionization because the compounds have basic properties. The ionization parameters are the temperature of ion source 200 °C, interface temperature 250 °C, and solvent cut time of 1.5 min. Based on the area, the optimum condition was acquired by using SIM mode with four (4) fragments consist of 2 fragments of MDEA and 2 fragments of Ephedrine HCl were chosen to use in this study.

System suitability test

This test was performed by injecting standard solution 5 times. The result was the percentage of coefficient variance (CV) below 2%. Thus, it met the requirement.

Optimization of blood spot volume and % hematocrit

After doing the system suitability test, GC-MS optimum condition has been obtained, which can be used to analyse MDEA in DBS sample. In DBS, various optimizations are performed to achieve the best possible conditions. The preparation was done without derivatization.

In this optimization, various amount of blood spot volume and % hematocrit were used. The amount of blood spot volume may affect the drawing capacity of the MDEA compound from the liquid-liquid extraction process carried out in this study. For hematocrit, the higher the hematocrit, the higher also the viscosity of blood. The 40 μl spot volume and 43% hematocrit has the largest area. Thus, it is the most optimal spot volume and % hematocrit to be used in this study. From these results, it can also be said that with a 40 μl spot volume, MDEA compound withdrawal capacity is better than that of other bottling volumes. The volume 50 μl was not used because the bottle exceeds the circle limit for the blood spot on the paper.

Optimization of reconstitution and extraction of solvent volume

From the result, the optimal reconstitution volume used is 50 μl viewing the resulting area larger than the 100 μl. This is due to the more concentrated 50 μl that can affect the detection process on the tool so that the analytical readings get better. For extraction solvent volume, 700 μl is the most optimum extraction solvent volume to be used in this extraction. It can be seen from the resulting area is larger than the other variations of volume. From this point, it can be said that with a volume of 700 μl the MDEA compound withdrawal capacity from DBS becomes larger.

Optimization of sonication duration

Based on the result, 5 min was chosen for sonication time based on consideration of the area obtained when compared with the time of sonication for 15 min and 30 min, which has different insignificance and efficiency of time required for extraction.

Below is the chromatogram of the final chosen optimization method.

Table 1: System suitability test using GC-MS

| No. | Area (μV/s) | Retention time (min) | PAR MDEA | Ephedrine HCl |
|-----|------------|---------------------|----------|---------------|
| 1   | 277319     | 149604              | 10.745   | 8.132         | 1.85   |
| 2   | 282774     | 152109              | 10.743   | 8.123         | 1.86   |
| 3   | 278393     | 148730              | 10.746   | 8.131         | 1.87   |
| 4   | 283773     | 148283              | 10.743   | 8.132         | 1.82   |
| 5   | 277196     | 152175              | 10.746   | 8.132         | 1.82   |
| mean±SD | 279891±3142.60 | 150180±1852.97      | 10.74±1.34 | 8.130±3.78   | 1.86±0.03 |
| CV (%)            | 1.12        | 1.23                | 0.01     | 0.05          | 1.79   |

Fig. 2: Mass spectrometry detection of MDEA on SIM mode

Fig. 3: Chromatogram of selected method optimization at a concentration of 100 ng/ml (700 μl extraction volume, 5 min sonication, 50 μl reconstitution volume)
**LLOQ measurement**

In LLOQ, the measurement was done in 5 replicates, LLOQ concentration of 15 ng/ml was obtained with \( \% \text{diff} \geq 1.14\% \) and CV at 8.63\%. From this result, it can be said that the measurement of LLOQ meets the criteria that \( \leq \pm 20\% \). After that, the concentration measurements were taken by half to 7.5 ng/ml. From the measurement result, the \% \text{diff} \ and CV values obtained do not meet the requirements; thus the final LLOQ value was 15 ng/ml. The obtained LLOQ value of 15 ng/ml also meets the requirements because it still not exceed 5\% maximal concentration (Cmax) of MDEA in the blood, which is 330 ng/ml \([15]\).

In the fig. 2 the chromatogram of MDEA at LLOQ concentration is shown.

**Linearity calibration curve**

The obtained linearity also meets the requirements with \( r \geq 0.98 \) with a concentration range from 15-250 ng/ml. The calibration curve was made consisting of blank samples (DBS without analyte and internal standard), zero samples (DBS with internal standard), and nonzero samples (DBS with an analyte and internal standard) of 15, 50, 75, 100, 150, 200, and 250 ng/ml. The analysis was performed by observed the linearity, \% \text{diff} \ \leq \pm 20\% \) for LLOQ, and \( \leq \pm 15\% \) for concentrations other than LLOQ.

The result of the data obtained meets the requirements with \% \text{diff} \ \leq \pm 20\% \) for LLOQ and \( \leq \pm 15\% \) concentrations other than LLOQ. The calibration curve yields a linear regression equation \( y = -0.0046 + 0.0020x \) with \( r = 0.9963 \) where \( x \) is the MDEA (ng/ml) concentration and \( y \) is the peak area ratio (PAR) between MDEA and internal standard.

The calibration curve was made every day when doing analysis. The purpose is to prevent the occurrence of measurement errors due to if the GC-MS condition changes between days. The calibration curve should meet the \( \leq \pm 20\% \) precision criteria for LLOQ and \( \leq \pm 15\% \) for other concentrations. From the measurement of the calibration curve between days, the value of CV meets the requirements and the correlation coefficient \( (r) \) averages 0.9954.

**Intra-day accuracy testing**

Intra-day accuracy testing results were \% \text{diff} \ range from 2.12 to 17.29\% for LLOQ and \% \text{diff} \ concentrations range from 4.63 to 14.33\% for QC concentrations. For precision, testing results were \%CV 8.86\% for LLOQ and range from 4.85 to 8.53\% for QC. The results of the accuracy testing results were \% \text{diff} \ range from -19.87 to 18.18\% for LLOQ and \% \text{diff} \ range from 14.19 to 14.33\% for QC concentrations. For precision, testing results were \%CV 14.16\% and range from 7.74 to 9.25\% for QC. Recovery of the method was 81.26 to 90.95\%.

**Table 2: Calibration curve of MDEA**

| Value (ng/ml) | 0.00 | 15.00 | 50.00 | 75.00 | 100.00 | 150.00 | 200.00 | 250.00 | R     | Slope  | Intercep |
|--------------|------|-------|-------|-------|--------|--------|--------|--------|-------|--------|-----------|
| Number       | 1    | 2     | 3     |       |        |        |        |        |       |        |           |
| Measured value (ng/ml) | 15.57 | 14.09 | 13.69 | 14.59 | 55.03  | 45.78  | 45.89  | 48.90  | 70.03 | 93.81  | 164.14  |
|               | 103.95| 78.25 | 68.96 | 72.41 | 132.12 | 98.18  | 88.61  | 95.46  | 199.11| 169.25 | 195.70   |
|               | 260.31| 188.74| 246.32| 253.18| 0.9954 | 0.9963 | 0.9943 | 0.9954 | 0.9954| 0.9963 | 0.9954   |
|               | 0.0017| 0.0020| 0.0022| 0.0020| -0.0065| -0.0046| -0.0177| -0.0096|       |        |           |
| mean±S       | 14.45±0| 48.90±5.3| 72.41±5.0| 95.46±7.8| 155.17±20.1| 195.70±6.0| 253.18| 0.9954| 0.0020| -0.0096|           |
| D            | 99    | 1     | 0     | 0     | 2      | 3      | ±7.00  |        |        |        |           |

**Limit of detection (LOD)**

In the Limit of Detection (LOD) measurements performed on the statistic by using calibration curve statistics, it was found to be 8.49 ng/ml.

**Accuracy precision and % recovery**

Accuracy precision was done in 5 replicates from each four concentrations (LLOQ, QCL, QCM, and QCH) 3 times at a minimum 2 d.

Fig. 4: Chromatogram of MDEA at LLOQ concentration and Ephedrine HCl as IS

Fig. 5: Chromatogram comparison between sample in LLOQ concentration and blank sample
Selectivity
In this parameter, it was performed using DBS samples of MDEA in LLOQ concentrations and DBS blank samples using six different blood sources. Selectivity parameter test aims to see if at the retention time of MDEA and standard in the presence of interference from blood extract in the form of % interference. The requirement is % interference ≤20% for LLOQ and ≤5% for IS. Based on the result, % interference were 6.06-9.49% for MDEA and 0.32-0.69% for ephedrine HCl. From this result, the method has met the validation requirement.

Matrix effect
Matrix effect is needed to be tested since the mass spectrometry was used for analysis. In this parameter, testing was performed by preparing 40 µl blood blot prepared and do the sample preparation. The supernatant obtained added MDEA at low concentrations (QCL) at 45 ng/ml and high concentrations (QCH) at 187.5 ng/ml. A final solution of 1 µl is injected into GC-MS under selected analysis conditions. After that, MDEA standard solutions are prepared at low concentrations (QCL) and high concentrations (QCH). Thereafter, a final solution of 1 µl was injected into GC-MS under selected analytical conditions. The above procedure was also done for internal standards. The matrix effect was seen by observing the matrix factor, i.e., comparing the peak area of MDEA and the internal standard in the blood and the peak area of MDEA and the internal standard solution. The requirement was % CV ≤1% for QCL and QCH. The result testing was the standardized, normalized matrix factor in 0.77 for QCL and 0.83 for QCH. Based on the result, it can be said that the autosampler stability test parameters meet the requirements with % diff ranges from ≤5.10% to 1.52% for QCL and ranges from 1.04% to 1.13% for QCH. From the results, it can be said that MDEA samples within the DBS can still be stored in the autosampler for 24 h before being injected into the tool.

Stability
Three types of stability test were done, stock solution at short and long term, DBS at short and long term, and autosampler at 0 h and 24 h.

Stock solution stability
The result of the stability test of MDEA and Ephedrine HCl stock solution can be said that % diff meets the requirement, which was below ≤6%, % diff for MDEA in the short term in room temperature range from 0.21% to 0.61% and ephedrine HCl in short term range from 0.09% to 0.11%. From this result, it can be said that MDEA stock solution and stock solution of Ephedrine HCl remain stable during storage in room temperature for 24 h.

In the long-term stability test results, on day 30, MDEA stock solutions had % diff from 0.65% to 1.25% and ephedrine HCl stock solution having % diff range from 0.14% to 0.21%. From the result, it can be said that MDEA stock solutions and stock solutions of ephedrine HCl remain stable during long-term storage for 30 d at 4°C.

DBS sample stability
This parameter is conducted by storing QCL and QCH samples at room temperature for 24 h and then observing the stability of the hours 0, 6, and 24 by observing % diff. The storage was carried out in a sealable plastic equipped with a desiccant. From the results obtained it can be said that the short term stability of MDEA in DBS meets the requirements of 7.97% to 8.81% for QCL samples and 2.30% to 9.67% for QCH samples. This indicates that MDEA samples within the DBS can be stored at room temperature for 24 h.

In long-term stability, samples were stored in sealable plastic equipped with a desiccant at temperature 4°C up until 21 d. % diff was observed by analyze at day 0 and day 21. From the results obtained it can be said that the long-term stability parameters of MDEA in DBS meet the requirements with % diff from 2.11% to 8.60% for QCL samples and 9.64% to 10.80% for QCH samples. From the results, it can be said that MDEA samples in DBS can still be stored in 4°C for 21 d because long-term stability still meets the requirements. This is also correlated with research conducted by Ambach et al. in 2013 that the DBS sample is stable for at least 2 w of storage at 4°C. The results of this stability test also become the advantages of using DBS samples that can be stored in the long term before the analysis is done [16].

Autosampler stability
The purpose of this test is to know the length of time to store samples in the autosampler until it can still be used for the analysis process after the extraction process is done. This test is performed by injecting QCL and QCH samples at 0 and 24 h. From the test result, it can be said that the autosampler stability test parameters meet the requirements with % diff ranges from 5.10% to 1.52% for QCL and ranges from 1.06% to 1.13% for QCH. From the results, it can be said that MDEA samples within the DBS can be stored in the autosampler for 24 h before being injected into the tool. Based on the result, it could be said that all stability test has fulfilled the criteria of validation.

Based on the results obtained, it can be said that the analytical method developed in this study has several advantages. The advantages are relatively faster preparation time that is by using sonication for 5 min compared to research conducted by Ambach et al. which in 2013, using vortex for 15 min [16]. The use of sonication may increase the solubility of the analyte to the extracting process when the extraction process is carried out. In addition, sonication can also provide a higher recovery [17]. Another advantage possessed is when compared with research by Westphal et al. in 2007 on the development of MDEA analysis method in serum using GC-MS after derivatization was LLOQ obtained in the current study that is 15 ng/ml not so much different from LLOQ in previous research that is 13.2 ng/ml [18]. In addition, in this study did not use derivatization compared with previous researches [19]. Research on MDEA with analysis using GC-MS is not widely practiced especially in narcotic analysis. This can be an advantage in this study considering that the instrument used is GC-MS and able to detect the compound up to a very small concentration [5].

The next advantage is the process of derivatization was not required. In the analysis using GC-MS usually use the derivatization process. Derivatization has a number of drawbacks that make longer procedural preparation steps and more costly, the data acquisition process becomes more complex and longer because derivatization can sometimes lead to impurities, the uncertainty of conversion of compounds into derivatives, the use of toxic reagents [19, 20]. The next advantage is that the preparation of the instrument is relatively easier for GC-MS than LC-MS/MS from the aspect of the mobile phase. GC-MS uses gas phase while in LC-MS/MS mobile phase used in the form of reagent or reagent mixture so it takes more time to prepare before doing analysis. In addition, the operational cost of the instrument can be cheaper for GC-MS than LC-MS/MS because in GC-MS, mass spectrometry analyzer used in the form of single quadrupole while in LC-MS/MS, using triple quadrupole which certainly cost more.

CONCLUSION
Based on the results, it could be concluded that the optimum condition of the analytical method by using GC-MS was obtained and has fulfilled validation criteria. The developed method was simple with liquid-liquid microextraction and without derivatization and was linear in concentration of 15-250 ng/ml.
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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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