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RESEARCH ARTICLE

Development of MDMA analysis method in dried blood spot using gas chromatography mass spectrometry Quadrupole Time-of-Flight

Aisyah Alifia Anjeli[1], Baitha Palanggatan Maggadani[1], Prisma Andini[2], Yuswardi[2]

[1] Faculty of Pharmacy, Universitas Indonesia, Depok, Indonesia
[2] Centre Forensic Laboratory, Criminal Investigation Agency, Indonesian National Police, Bogor, Indonesia

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Correspondence
Baitha Palanggatan Maggadani
Faculty of Pharmacy
Universitas Indonesia
Depok
West Java
Indonesia
baitha.p@farmasi.ui.ac.id

Abstract

Background: 3,4-Methylenedioxymethamphetamine (MDMA) or ecstasy is a synthetic stimulant of the Central Nervous System (CNS) that induces hallucinogenic effects, addiction, and drowsiness. To minimise MDMA misuse, accurate testing is needed using the most reliable specimen, which is blood. **Objective:** This study aims to develop an MDMA analysis method using dried blood spot (DBS) sampling, optimise the analysis conditions, prepare the DBS samples, and validate the analytical process. **Method:** MDMA was analysed using gas chromatography-mass spectrometry, Quadrupole Time of-Flight, with ephedrine HCl as an internal standard. DBS preparation involved applying 30 μL of blood, drying for 3 hours, and extracting with methanol, followed by vortexing, sonication, and reconstitution with ethyl acetate. **Result:** The method validation was performed based on the FDA 2022 M10 Bioanalytical Method Validation and Study Sample Analysis guidelines, showing linearity within the concentration range of 15.00 200 ppb. **Conclusion:** The optimised method demonstrated reliable results for the analysis of MDMA in DBS samples, offering a simplified, less invasive blood collection technique with high analytical precision.

Introduction

3,4-Methylenedioxymethamphetamine (MDMA), which is also popularly known as ecstasy, is a synthetic central nervous system stimulant that can lead to addiction, hallucinations, blurred vision, and drowsiness (NIDA, 2024). Classified as a Schedule I narcotic under Indonesian Law No. 35/2009, MDMA is prohibited for medical use and is only allowed for research purposes. Despite strict regulations, MDMA misuse remains prevalent in Indonesia, with over 1.6 million tablets seized in 2023, making it one of the most commonly abused drugs (National Narcotics Agency, 2023).

Forensic analysis of MDMA requires accurate detection methods using biological specimens such as blood, urine, and hair. While urine tests can be easily tampered with and take time to yield results, hair samples are costly and complex to analyse. Blood is the most reliable specimen, but conventional collection methods, like venipuncture, can be invasive and uncomfortable (McNeil, 2023).

Sampling of Dried Blood Spots (DBS) has emerged as a less invasive alternative, enabling the easier collection of small blood volumes by pricking the finger or heel. This method is cost-effective and preserves sample integrity, making it suitable for forensic applications (Ghosh *et al*., 2023). Previous research has shown that DBS can effectively detect New Psychoactive Substances (NPS), and this study aims to optimise a method for analysing MDMA using DBS in conjunction with gas chromatography-mass spectrometry Quadrupole Time-of-Flight (GC/MS Q-ToF). The goal is to establish a sensitive, reliable detection method for MDMA in blood, aiding law enforcement and rehabilitation efforts in Indonesia (Harahap *et al*., 2020).

This study aims to develop an optimised method for analysing MDMA in DBS samples using gas GC/MS Q-

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ToF, providing a sensitive and reliable approach for detecting small concentrations of MDMA in the blood of users. This method is expected to enhance forensic applications in Indonesia.

Methods

Research tools

The research tools included GC/MS Q-Tof 7250 (Agilent, USA), Nitrogen Generator Compressor (PEAK Scientific, UK), Nitrogen Evaporator XcelVap (Horizon Technology, USA), HP-5 MS Column (5.30 m x 0.32 mm, 0.25 µm) (Agilent, USA), Mass Analyzer data processing system (Agilent, USA), computer (HP, USA), Microtube ThermoMixer® C (Microtube, Germany), digital analytical balance with Xpe205 sensor (Mettler Toledo, Switzerland), centrifuge (Thermo Fisher Scientific, USA), SONICA® Ultrasonic Cleaner (Soltec, Italy), Vortex Genie 2 (Scientific Industries, USA), micropipettes (10-100 µl and 100-1000 µl sizes) (Transferpette, Merck, Germany), fume hood (Duraline, Singapore), microcentrifuge tubes, autosampler vials, and autosampler vial inserts (Agilent, USA).

Research materials

The research material included racemic standard (±) 3.4-Methylenedioxymethamphetamine, 1.000 ppm in methanol solvent (Cerilliant, Germany), Ephedrine HCl Standard (Indonesian Pharmacopoeia Comparative Standard), DBS paper (Whatman, UK), micropipettes (sizes 10 µl, 200 µl, and 1000 µl), Parafilm (Bemis, USA), whole blood samples (Indonesian Red Cross, Jakarta, Indonesia), methanol for analysis, ethyl acetate, and helium gas 99.99% (Merck, Germany).

Preparation of stock solution of Ephedrine HCl as an internal standard

The preparation of a stock solution of Ephedrine HCl is created by dissolving the standard substance into methanol to obtain a concentration of 1000 ppm. Then, 10 mg of Ephedrine HCl standard is scaled and placed in a volumetric flask sized 10 ml. Methanol is then added to the flask to a volume of approximately half its total volume, and the compound is shaken until it is completely dissolved. Next, methanol is added to the calibration mark, and the solution is shaken gently until it becomes homogeneous.

Preparation of calibration curve solutions

The calibration curve standards are prepared by diluting a specific volume of the MDMA stock solution (1000 ppb) with methanol to obtain the appropriate working standard solutions. Each concentration is then diluted with blood to achieve calibration curve standards with MDMA concentrations of 15, 30, 75, 90, 150, and 200 ppb.

Preparation of quality control samples (QC Samples)

Samples for the quality control are created by diluting the MDMA stock solution 1000 ppm. The dilution is designed to achieve specific concentrations for the quality control samples. The dilution is carried out to obtain three concentrations: QCL (3 times the Lower Limit of Quantification (LLOQ)), QCM (30-50% of the calibration curve range), and QCH (minimum 75% of the Upper Limit of Quantification (ULOQ)). The provisional LLOQ based on the calibration curve standards is 15 ppb.

System suitability test

The system suitability test ensures that the optimum conditions provide good repeatability. A mixture of MDMA and Ephedrine HCl solutions (5 µg/ml) is injected into the device (1 µl) under optimum analysis conditions, which are a temperature of the column of 250 °C and a flow rate of 1.2 ml/minute. Five replicates are injected using electron ionisation mode and complete scan of detection, and the coefficient of variation (CV) is assessed for the area ratio and retention time of the analyte and internal standard. The acceptance criteria are a %CV of ≤ 2%.

Sample preparation

The sample preparation was carried out by diluting MDMA in blood to obtain a specific concentration (Table I). Blood containing MDMA was then dropped, 30 µl in volume, onto DBS paper that had been prepared using an optimised sample preparation method. The sample was added to a standard solution of 5 ppb Ephedrine HCl and a selected volume of extraction solution, then vortexed for a specified duration. The sample was then sonicated for a designated time and centrifuged at 5000 rpm for the chosen duration. The supernatant was collected and dried under a stream of N_2 gas for 30 minutes until dry, and restored with 100 µl of ethyl acetate. The extraction was repeated to obtain a cleaner result by vortexing for 30 seconds, sonication for 10 minutes, and centrifugation at 5000 rpm for 5 minutes. The supernatant was split and placed into a GC vial, and 1 µL was injected into the instrument under the chosen conditions.

Table I: Variation of analytical conditions and sample preparation optimisation

Optimisation	Parameter	Variation	Optimum condition
Analytical condition	Flow rate	0.8; 1.0; 1.2 ml/minutes	1.2 ml/minutes
	Column temperature	250°C; 280°C; 300°C	250°C
DBS* sample preparation	Extraction solvent volume	500 µl; 750 µl; 1000 µl	500 µl with Methanol
	Sample spot volume	10 µl; 20 µl; 30 µl	30 µl
	Sample drying time	1; 2; 3 hours	3 hours
	Vortex time	30; 60; 90 seconds	90 seconds
	Sonication time	5; 10; 15 minutes	10 minutes
	Centrifuge time	5; 10; 15 minutes	10 minutes

*DBS: Dried Blood Spot

Validation assay

The developed method underwent complete validation assay organised in the form of LLOQ measurements, calibration curve, accuracy, linearity, precision, selectivity, matrix effect, recovery, carryover, dilution integration, stability, and reinjection reproducibility using DBS Whatman™ 903 Protein Saver Card samples based on the US Food and Drug Administration (2022) guidelines adopted from the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) M10 guidelines (US FDA, 2022).

Results

Figure 1 illustrates the mass spectrum results obtained for MADMA. Additionally, Figure 2 presents the chromatogram corresponding to the selected method optimisation conducted at a concentration of 1 ppm.

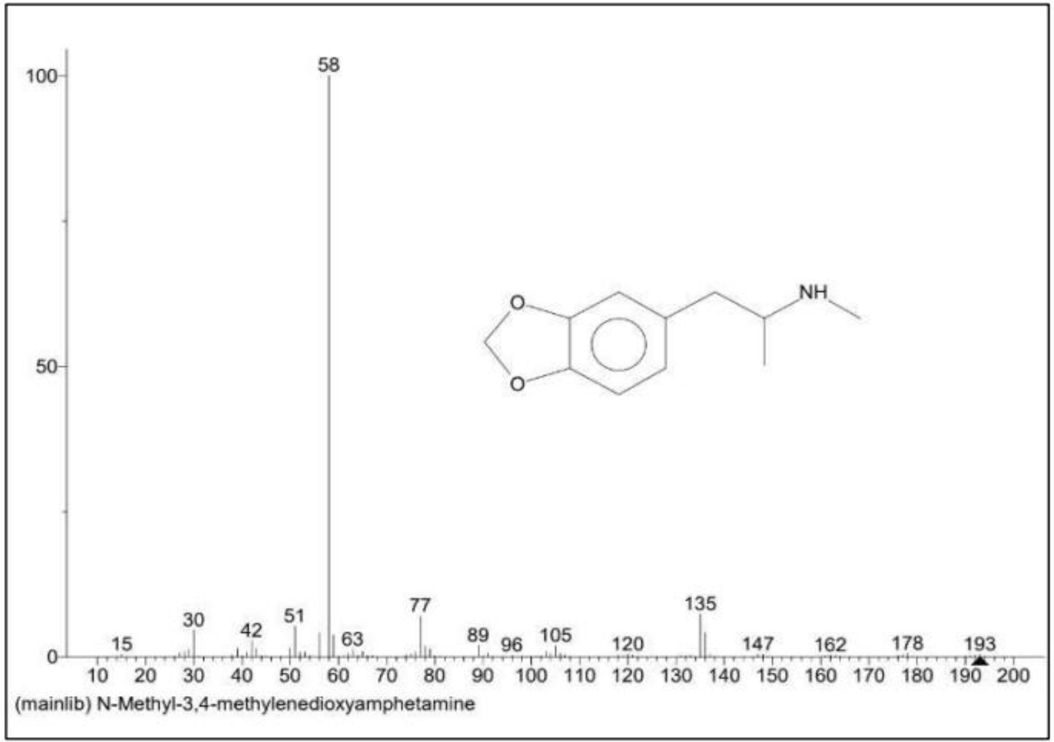

Figure 1: Mass spectrum of MDMA

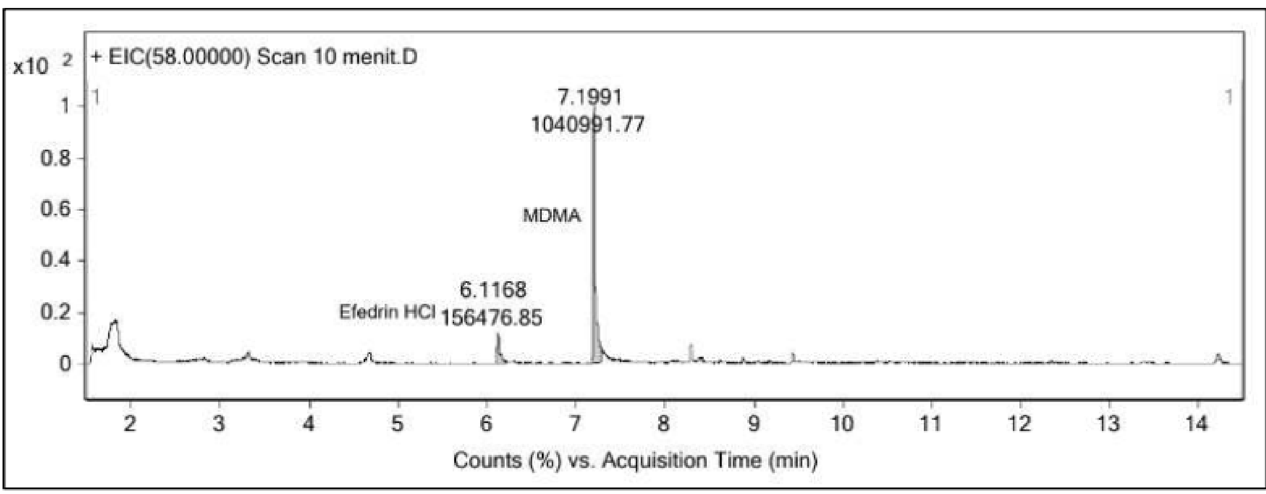

Figure 2: Chromatogram of selected method optimisation at a concentration of 1 ppm

Discussion

The extraction method and analysis conditions were validated by testing the analytical validation parameters in accordance with the method validation guidelines for bioanalysis from the US Food and Drug Administration (US FDA), 2022. Several parameters of the validation that must be fulfilled are the lower limit of quantification (LLOQ), dilution integrity, selectivity, matrix effect, linearity and calibration curve, recovery, accuracy and precision, reproducibility of repeat injections, carry-over, and stability. The MDMA concentration of 15 ppb was set as the LLOQ.

When compared to a study conducted using this method, it was able to produce a lower quantification limit for MDMA, at 7.5 ppb, although it employed a more sensitive and superior HPLC-MS/MS instrument for quantitative analysis. In contrast, the study by Harahap *et al.* (2020) produced a higher quantification limit of 25 ppb using GC-MS instruments.

By diluting the standard solution in a whole blood sample to a concentration of 1000 ppb, the calibration curve for the stock solution is then created. Further serial dilutions were made to the specified concentrations. A calibration range of 15-200 ppb was achieved with six concentration points: 15, 30, 75, 90, 150, and 200 ppb, along with a blank and zero sample. The correlation coefficient for this concentration range was 0.9981, with a linear regression equation of -0.1081x + 0.123 and an R-squared value of 0.9981. The result of the calibration curve was successive and matched the qualification, i.e., the percentage difference (%diff) was not more than 15% at every concentration, except for the LLOQ, which was no greater than 20%.

The test results demonstrated that endogenous components in blank sample matrices from six different whole blood sources did not interfere with the analysis. These samples met the ICH FDA 2022 requirements, as the response generated did not exceed 20% of the analyte area at LLOQ concentration and remained below 5% of the internal standard area. Whole blood samples were tested from different blood groups to ensure these parameters did not affect the analysis. Interference responses on MDMA ranged from 0.84% to 6.42%. Meanwhile, for the internal standard ephedrine HCl, the interference response ranged from 0.02% to 0.90%.

Accuracy and precision tests were performed by assembling five duplicate quality control samples at four dissimilar concentration levels: LLOQ, QCL, QCM, and QCH. For MDMA, these concentrations were 15 ppb, 45 ppb, 92.5 ppb, and 150 ppb, respectively.

Testing was conducted both within-run (in a single analysis) and between-run (on different days) to determine whether the concentration remained the same after repeated injections on the same day or on a different day, compared to the actual concentration. Accuracy, which refers to the proximity between the weighed concentration used inside the analysis and the exact concentration, was represented as %diff.

Precision, which refers to the repeatability of the analyte measurements, was represented by the %CV. The results showed that the within-run accuracy and precision for MDMA met the requirements with %diff values for LLOQ, QCL, QCM, and QCH ranging from: 6.08% to 11.82%; -9.20% to -5.78%; -0.76% to 3.76%; and -8.98% to -0.55%, respectively, and %CV values of: 2.43%; 3.53%; 5.51%; 4.23%, respectively. The between-run accuracy and precision results for MDMA

also met the requirements, with %diff and %CV values for LLOQ, QCL, QCM, and QCH concentrations within ±15%. Additionally, the %CV values for each concentration were also within the acceptance criteria.

Recovery tests were conducted both relatively and absolutely. A comparison process was conducted between the area of the non-extracted analyte and the area of the extracted analyte, both of which were at the same concentration, to calculate the recovery. The percentage recovery for MDMA ranged from 85.54% to 96.49%, while for ephedrine it ranged from 92.47% to 93.96%.

Carry-over analysis showed that analyte carry-over in subsequent injections ranged from 1.835% to 2.077%. For the internal standard ephedrine HCl, carry-over percentages ranged from 0.034% to 0.054%. From the data, it was concluded that the analyte carry-over in subsequent injections was minimal and met US FDA (2022) requirements.

Dilution integrity testing was performed to verify that sample dilution did not affect the accuracy and precision of the results. The test was performed in five replicates using concentrations of 2x QCH (300 ppb), QCH (150 ppb), and ½ QCH (75 ppb). The acceptance criteria for dilution integrity testing were that %diff and %CV values should not exceed ±15%. The test results for the 2x QCH concentration showed %diff values ranging from -13.50% to -5.35% with a %CV of 6.99%. For the QCH concentration, %diff ranged from -11.76% to 6.29% with a %CV of 7.59%. For the ½ QCH concentration, %diff ranged from -13.46% to -4.87% with a %CV of 7.19%. The results indicated that dilution integrity testing did not affect accuracy and precision, and all criteria met the guidelines from ICH FDA 2022.

Matrix effect testing was conducted to determine whether a matrix effect on the biological properties of the samples could be identified. Testing was performed on six blank matrices from different sources at QCL and QCH concentrations. From this test, both analytes met FDA (2022) requirements; the %diff and %CV obtained from these 6 sources did not exceed ±15%.

Stability testing was conducted using stock solutions of MDMA and ephedrine, which were evaluated for a minimum of 24 hours at 25°C and a minimum of 14 days at -20°C. DBS samples were tested for stability for at least 24 hours at 25°C and at least 14 days at -20°C. Processed samples were stable for a minimum of 24 hours at 15°C in an autosampler. All stability tests demonstrated acceptable results and met the applicable guidelines.

Reinjection reproducibility was evaluated to investigate whether reinjections affect accuracy and precision. Testing was carried out by re-injecting the previously used samples after storing them for 24 hours. The used sample caps were replaced with new ones prior to reinjection. Testing was conducted at QCL, QCM, and QCH concentrations, each in five replicates. Based on the results, it can be concluded that the samples remained stable after 24 hours, even after initial analysis.

Conclusion

Results from the method validation have been done in the form of LLOQ measurements, selectivity, recovery, linearity calibration curve, accuracy, dilution integration, precision, carryover, matrix effect, stability, and reinjection reproducibility. The method for MDMA analysis in DBS using GC/MS Q-Tof has been validated and meets the acceptance criteria outlined in the FDA's 2022 M10 Bioanalytical Method Validation and Study Sample Analysis requirements.

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