Research Article

DEVELOPMENT AND VALIDATION OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE ANALYSIS METHODS IN DRIED BLOOD SPOT SAMPLE USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Yahdiana Harahap ^{*1}, Ricky Julio ¹, Kuswardani ²

¹Faculty of Pharmacy, University of Indonesia, Depok, West Java 16424, Indonesia

²Narcotics Laboratory, National Narcotics Board, Bogor, Indonesia, Bogor

*Corresponding Author Email: yahdiana03@yahoo.com

Article Received on: 05/03/19 Approved for publication: 02/06/19

DOI: 10.7897/2230-8407.1007212

ABSTRACT

Objective: This research was carried out to obtain the MDMA bioanalysis method in DBS using liquid-liquid micro-extraction method using gas chromatography mass spectrometry (GC-MS) with the standard in Ephedrine. **Methods:** The blood was spotted on DBS paper and dried prior to analysis. The instrument used is Shimadzu GCMS-QP2010 Ultra Mass Chromatography Gas, with an HP-5 MS capillary column length of 30 m, 0.25 mm diameter and a film thickness of 0.25 μ m. The mobile phase used is 99.99% Helium gas and split less injection mode. **Results:** The optimum chromatography condition for MDMA analysis in DBS is the HP-5 MS capillary column with a length of 30 m; inner diameter 0.25 mm; Helium mobile phase 99.999%; flow rate of 0.8 mL / minute; MS detection at m / z 58.00 and 135.00 and ephedrine HCl as internal standard. Sample preparation used a liquid-liquid micro-extraction method with methanol as solvent then it was sonicated or 15 minutes then dried with Nitrogen gas and reconstituted with 40 μ L of ethyl acetate then centrifuged for 5 minutes with 3000 rpm. **Conclusion:** The developed method for determination of MDMA in DBS fulfilled validation based on EMEA bioanalytical guidelines in 2011 for linearity, LLOQ limits, accuracy and precision, selectivity, carry over, matrix effect and stability. The method obtained was linear in the concentration range 25.0-500.0 ng / mL with $r > 0.999$.

Keywords: ephedrine HCl, DBS, GC-MS, MDMA, optimization, validation

INTRODUCTION

3,4-Methylenedioxymethamphetamine (MDMA) or known as ecstasy is one of the most misused drugs in the world, including in Indonesia.¹ MDMA belongs to the class of stimulants and hallucinogens^{2,3}. The method for analyzing MDMA in DBS samples by tandem gas chromatography of mass spectrometry is still limited. Based on the research that has been done, it is known that the maximum concentration of MDMA in plasma can reach until 331 ng / ml after 2 hours of dosing with 2 ng / ml LOD in whole blood and 1.6 ng / ml in DBS with a volume of 100 μ L using High Performance Liquid Chromatography. In some literature studies, it is stated that during this time to identify MDMA abuse urine samples were used because it was a non-invasive sampling technique. However, the feasibility of collecting urine samples is being debated in the case of sampling surveillance.⁴ As a result of concerns in privacy violations in sampling surveillance, the truth process from the sample can still be doubted. This research will be carried out to obtain the MDMA bioanalysis method in DBS using liquid-liquid micro-extraction method using gas chromatography mass spectrometry (GC-MS) with ephedrine HCl as internal standard. This method is expected to be applied for forensic laboratory tests against misuse of MDMA.

MATERIALS AND METHODS

Samples were taken using the Spike method, namely by using blood control that has been determined according to the study concentration. As much as 40 μ L of blood was spotted on Perkin Elmer DBS paper then dried for 3 hours. After drying the paper,

cut the diameter and then dissolved with 500 μ L ethanol and added 100 μ L of ephedrine HCl as internal standard, then dried under nitrogen gas flow to dry. After drying, then reconstituted with 40 μ L of ethyl acetate which was added with 10 μ L of 0.25% HCl. Next, the solution was centrifuged for 5 minutes then injected into the GC-MS system. The instrument used was Shimadzu GCMS-QP2010 Ultra Mass Chromatography Gas (BNN, Bogor), with an HP-5 MS capillary column with a length of 30 m, an inner diameter of 0.25 mm and a film thickness of 0.25 μ m. The mobile phase used was 99.99% Helium gas and splitless injection mode. Materials used were standard MDMA (Cerrilant analyte), standard in ephedrine HCl (National Agency of Drug and Food Control, Indonesia), methanol (Merck), ethyl acetate (Merck), hydrochloric acid (Merck), and blood samples from the Indonesian Red Cross (PMI). The calibration curves used were at 25, 50, 75, 100, 250, 500 ng / mL.

RESULT AND DISCUSSION

The resulting LLOQ was 25 ng / ml with a calibration curve of 25, 50, 75, 100, 250, 500 ng / ml with linearity $r = 0.9999$ with a linear regression equation $0.0010x + 0.0029$. The QCL used was 75 ng / ml, the QCM used was 263 ng / ml, and the QCH used was 375 ng / ml. Accuracy and precision both within run and between run meet EMEA 2011 requirements. Recovery is obtained from the comparison of the area of the extracted analyte and the area of the unextracted analyte with the same concentration. The percentage of recovery was 82.31% to 96.76%. Based on EMEA 2011 the recovery test value is not required⁷, but in FDA Guideline 2018 it is stated that the recovery percentage does not have to be 100% but must meet must be

consistent, precise and reproducible.⁵⁻⁶ Selectivity was carried out and produced an interference response on MDMA ranging from 5.03% until 10.78% and on ephedrine HCl, an interference response of 0.00% was obtained. Carry over is carried out to see the percentage of analyte in the blank after high concentration injection. The percentage of analyte ranged from 12.09% while ephedrine HCl was 0.00%.

CONCLUSION

The validation results of the MDMA analysis method in DBS were fulfilled validation based on EMEA bioanalytical guidelines in 2011 that fulfills the criteria for linearity, LLOQ limits, accuracy and precision, selectivity, carry over, matrix effect and stability. The method obtained was linear in the concentration range 25.0-500.0 ng / mL with $r > 0.999$.

Table 1 : The Result of LLOQ Test

Conc.	Area (μ V.s)		PAR		Measured Concentration			
(ng/mL)	MDMA	IS		Kons. Terukur (ng/mL)	Rata - rata (ng/mL)	SD	% KV	% diff
25.00	14688	457975	0.0321	28.21	27.49	1.21	4.40	12.85
	14017	433064	0.0324	28.50				13.99
	15160	479493	0.0316	27.77				11.09
15.00	16789	535500	0.0314	27.52	27.76	5.02	18.07	10.06
	16560	567191	0.0292	25.43				1.73
	12358	431625	0.0286	24.89				65.90
	18655	464855	0.0401	36.00				140.02
	12286	447994	0.0274	23.72				58.13
	8208	249615	0.0329	29.00				93.30
	10743	371035	0.0290	25.20				67.98

Table 2 : The Result of Calibration Curve Test

Conc.	Area (μ V.s)		PAR	Measured Conc.	%Diff
(ng/mL)	MDMA	IS		(ng/mL)	
0.00	0	140043	0.0000	-2.79	0.00
25.00	14017	433064	0.0324	28.50	13.99
50.00	24781	454877	0.0545	49.87	-0.25
75.00	36864	439872	0.0838	78.22	4.30
100.00	42356	411966	0.1028	96.60	-3.40
250.00	151659	581971	0.2606	249.13	-0.35
500.00	367442	705821	0.5206	500.47	0.09
Slope (b)		Intercept (a)	R		R ²
0.0010		0.0029	0.9999		0.9998

Figure 1: The Result of Chromatogram MDMA and Ephedrine HCl

Figure 2: The Calibration Curve of MDMA and ephedrine HCl

ACKNOWLEDGMENT

The authors acknowledge Directorate of Research and Community Engagement (DPRM) Universitas Indonesia for financial support

REFERENCES

1. National Anti Narcotic Agency.. Final Report of the National Survey on the Development of Drug Abusers, Fiscal Year 2014.. Jakarta: BNN, 2014.
2. Kreek, M., Schlussman, S., Bart, G., Steven LaForge, K., & Butelman, E. (2004). Evolving perspectives on neurobiological research on the addictions: celebration of the 30th anniversary of NIDA. *Neuropharmacology*
3. Weisheit, R. & White, W. (2009). *Methamphetamine: Its History, Pharmacology and Treatment*. Center City, Minn.: Hazelden. Williams & Wilk
4. Kang, J. (2012). *Principles and Applications of LC-MS / MS for the Quantitative Bioanalysis of Analytes in Various*

Biological Samples. *Tandem Mass Spectrometry - Applications and Principles*.

5. Jantos, R., Veldstra, J., Mattern, R., Brookhuis, K., & Skopp, G. (2011). Analysis of 3,4-Methylenedioxymetamphetamine; Whole Blood Versus Dried Blood Spots, *Journal of Analytical Toxicology*, 35(5), 269-273.
6. European Medicines Agency. (2011). *Guideline on Bioanalytical Method Validation* (Vol. 44). London: An agency of the European Union.
7. Food and Drug Administration., 2018. *Guidance for Industry Bioanalytical Method Validation*. United States of America: Department of Health and Human Services.

Cite this article as:

Yahdiana Harahap *et al.* Development and validation of 3,4-methylenedioxymethamphetamine analysis methods in dried blood spot sample using gas chromatography-mass spectrometry. *Int. Res. J. Pharm.* 2019;10(7):24-26 <http://dx.doi.org/10.7897/2230-8407.1007212>

Source of support: Directorate of Research and Community Engagement (DPRM) Universitas Indonesia, Conflict of interest: None Declared

Disclaimer: IRJP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IRJP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IRJP editor or editorial board members.