Pharmacy Education (2025) 25(2) 1 - 7
https://doi.org/10.46542/pe.2025.252.17

ICOPMAP SPECIAL EDITION

RESEARCH ARTICLE

Development of a 3,4-methylenedioxymethamphetamine analysis method in dried blood spot using ultra-high performance liquid chromatography quadrupole–Time of flight

Nadya Ayu Anandita[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

Keywords
3,4-Methylenedioxymethamphetamine
Dried blood spot
Ecstasy
UPLC-QToF

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

Abstract

Background: 3,4-Methylenedioxymethamphetamine (MDMA), or ecstasy, is one of the most abused narcotics in Indonesia. Biological samples can be used to analyse MDMA levels in the body. Urine sampling cannot be supervised for privacy reasons, and it raises doubts about the authenticity of the evidence compared with blood samples. Dried blood spot (DBS) is simpler and minimally invasive than conventional blood sampling. **Objective:** This study aimed to develop a validated MDMA analysis method in DBS using UPLC-QToF. **Method:** ESI+ mode was used with Acquity® UPLC BEH C18 column (2.1 x 100 mm; 1.7 µm); flow rate 0.10 ml/minute; mobile phase 5 mM NH_4HCOO/0.1% HCOOH and methanol (70:30); column temperature 50°C, with an injection volume of 5 µL. Quantitation was carried out at m/z 163.0789 for MDMA and m/z 148.1153 for ephedrine HCl (IS). **Result:** The LLOQ value obtained was 20 ng/ml for MDMA with a calibration curve range of 20 – 500 ng/ml. **Conclusion:** The analytical method complied with the requirements set by the US FDA (2022) and was adopted from ICH M10 guidelines.

Introduction

The 3,4-methylenedioxymethamphetamine, or MDMA, or ecstasy, is in the amphetamine group of narcotics that is ranked second as the most abused drug in Indonesia. The National Narcotics Agency, together with the Indonesian National Police, carried out supply and demand reduction to handle and prevent the rise of drug abuse cases. Supply reduction was carried out by arresting dealers and abusers, accompanied by seizing and examining evidence in the form of raw materials and biological samples. Demand reduction was carried out by early drug abuse detection using biological samples in various society groups (The National Narcotics Agency, 2023).

Urine remains the primary biological sample used as evidence by the National Narcotics Agency and the Indonesian National Police because of the non-invasive sampling method. However, urine sampling cannot be supervised for privacy reasons, which raises doubts about the authenticity of the evidence (Harahap *et al*., 2020). Another problem with urine sampling is the lack of public restrooms during raids. A biological sample that is unlikely to be altered is blood. Blood sampling is carried out under supervision. However, the conventional blood sampling method is invasive, requires a large sample quantity, and may cause discomfort and inconvenience during transportation because blood samples must be frozen before analysis in the laboratory (Maggadani *et al*., 2021). To overcome this problem, the dried blood spot (DBS) may be used as an alternative. DBS is a minimally invasive sampling method because the samples are taken through peripheral blood vessels, usually from the fingertips, and the amount of blood taken is relatively small (Denniff & Spooner, 2014; Maggadani *et al*., 2021).

While liquid blood samples have a short shelf life and usually need to be tested immediately after collection, DBS samples, which are in the form of dried drops of blood on paper, have a relatively long shelf life ranging from weeks to years (Gerostamoulos & Schumann, 2023).

The narcotics analysis method with DBS has been widely developed using stable isotope-labelled (SIL) internal standards (Odoardi *et al*., 2014; Kyriakou *et al*., 2016; Simões *et al*., 2018). However, these standards are relatively expensive and less applicable for routine examinations in developing countries such as Indonesia. Analogue compounds, such as ephedrine HCl for the amphetamine group, can be used as internal standards (Harahap *et al*., 2020).

Due to the low concentration of analytes in dried blood spots (DBS), ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QToF) is beneficial because of its high accuracy and sensitivity in mass analysis. A QToF mass spectrometer produces high-resolution mass spectra and offers excellent specificity, making it suitable for screening and identification (Pope *et al*., 2021). Additionally, QToF can also be employed for quantitative analysis. It allows simultaneous qualitative and quantitative assessments (Allen & McWhinney, 2019).

Methods

Reagents, standards, and chemicals

A 1.0 mg/ml (±) 3,4-Methylene-dioxymethamphetamine (MDMA) in methanol solvent (Cerilliant, Sigma-Aldrich, Germany); ephedrine HCl (Indonesian Food and Drug Authority, Indonesia); whole blood (Indonesian Red Cross, Jakarta, Indonesia); Whatman™ 903 Protein Saver Card (Cytiva, USA); methanol gradient grade for liquid chromatography, acetonitrile hyper grade for LC-MS, formic acid (HCOOH) 98 – 100% for HPLC (Merck Millipore, Germany); ammonium formate (NH_4HCOO) for mass spectrometry (Sigma-Aldrich, USA); ultra-high purity argon (PT Aneka Gas Industri, Indonesia); and ultrapure water (Arium® Pro Ultrapure Water Systems, Sartorius, Germany).

Preparation of solutions and standards

To create working standard solutions with concentrations of 10,000 ng/ml, 1,000 ng/ml, and 100 ng/ml, a stock solution of MDMA at 1.0 mg/ml was diluted with methanol. A blood sample was then used to dilute each concentration of the standard solution further, resulting in calibration solutions with blood MDMA concentrations of 20, 30, 50, 100, 200, 300, and 500 ng/ml. Quality control samples were prepared by diluting the working standard solutions with blood to achieve MDMA concentrations of 60, 200, and 400 ng/ml.

An ephedrine HCl stock solution was also prepared by dissolving 25.0 mg of ephedrine HCl (BPFI) in methanol within a 25.0 ml volumetric flask. The solution was sonicated for ten minutes to ensure it was fully dissolved.

UPLC-QToF procedure

The UPLC-QToF system is a UPLC H-Class unit consisting of Sample Manager FTN Acquity®; Quaternary Solvent Manager Acquity®; Acquity® UPLC BEH C18 Column (2,1 x 100mm; 1.7 µm); ESI Ion Source ZsprayTM; Mass Analyser Xevo G2-S QToF; data processed with MassLynx 4.1 Software (Waters, USA).

The Xevo G2-S QToF was in sensitivity mode with positive electrospray ionisation (ESI+). Mass spectrometry data were collected in a m/z range of 40 to 270 Da, using a capillary voltage of 3.00 kV and a cone voltage of 40 V. High-purity argon (99.999%) served as the collision gas, with a source temperature of 140°C and a desolvation temperature of 350°C, while the desolvation gas flow rate was 800 L/h.

Mass calibrations were performed in the m/z range of 40 to 280 using a 0.5 mM sodium formate solution in a 90:10 mixture of 2-propanol and water, infused at 20.00 µL/min with the collision energy off. The LockSpray setup used a lock mass solution of 1 ng/µL leucine enkephalin in a 50:50 acetonitrile-water mixture with 0.1% HCOOH, infused at 5.00 µL/min every 30 seconds. The monitored ion was the protonated fragment of leucine enkephalin at m/z 120.0813 Da, with a collision energy of 45 V.

Mass data was acquired in MS/MS mode (parallel reaction monitoring, PRM), with a mass range of 40 – 270 m/z. A lock-spray solution was used to correct. QToF acquired data in two functions for MDMA and ephedrine (IS). Target enhancement was set in m/z of the parent ion of each compound: 194 m/z for MDMA (Odoardi *et al*., 2014) and 163 m/z for ephedrine (IS) (Lee *et al*., 2021). A collision energy of 15 V was applied in both functions with a 40 V cone voltage. Quantification was carried out on the base peaks of both compounds.

Analytical condition optimisation

Several factors, such as the eluent combination, flow rate, and column temperature, were optimised for analytical conditions. The assay was performed using

MDMA and ephedrine (IS) standard solutions at concentrations of 300 and 500 ng/ml, respectively. Exactly 5 µL was injected into UPLC-QToF. The elution was run with an isocratic program, and the variations used for the eluent combination were 5 mM NH_4HCOO/0.1% HCOOH – acetonitrile (90:10); 5 mM NH_4HCOO/0.1% HCOOH – acetonitrile (80:20); 5 mM NH_4HCOO/0.1% HCOOH – methanol (80:20); and 5 mM NH_4HCOO/0.1% HCOOH – methanol (70:30). The variations used for flow rate optimisation were 0.1; 0,2; 0.3 ml/min. The variations used for column temperature were 30, 40, and 50°C. Retention time, area, and peak shape were used to compare the outcomes and evaluate the selected conditions.

System suitability test

A system suitability test was performed using a mixture of MDMA and ephedrine (IS) standard solution at concentrations of 300 and 500 ng/ml, respectively, injected 5 µL into UPLC-QToF under optimised conditions. The injection was done in five replicas. System suitability was evaluated by observing the coefficient of variation (%CV) of the peak area and the analyte and internal standard retention times. The %CV requirement was <2.0% (Epshtein, 2020).

Sample preparation optimisation

DBS sample preparation utilised the protein precipitation extraction method, optimising several conditions, including extraction solvent, blood spot volume, drying time, vortex time, sonication time, and centrifugation time. The optimisation included extraction solvents such as methanol, methanol with 0.1% HCOOH, and a methanol-acetonitrile (3:1 v/v) mixture. Solvent volumes were varied at 500, 750, and 1000 µL, while blood spot volumes were 10, 20, and 30 µL. Drying times were set to two, three, and four hours; vortex times to 30, 45, and 60 seconds; sonication times to five, ten, and fifteen minutes; and centrifugation times to five, seven, and ten minutes. Retention time, peak area, and peak shape were analysed to determine the optimal conditions.

Validation assay

A full validation assay was conducted using samples from the DBS Whatman™ 903 Protein Saver Card, by the guidelines set by the US Food and Drug Administration (FDA, 2022), which are based on the International Council for Harmonisation's (ICH) M10 guidelines (FDA, 2022). The validation parameters tested included selectivity, the lower limit of quantification (LLOQ), calibration curve, accuracy, recovery, precision, carryover, matrix effect, dilution integrity, reinjection reproducibility, and stability.

Results

The obtained optimised analytical conditions were a flow rate of 0.10 ml/minute, mobile phase 5 mM NH_4HCOO/0.1% HCOOH and methanol (70:30); column temperature 50°C, and an injection volume of 5 µL. The analysis was carried out with an Acquity UPLC® BEH C18 Waters column with a dimension of 2.1 × 100 mm × 1.7 µm. Quantitation was carried out at m/z 163.0789 for MDMA and m/z 148.1153 for ephedrine HCl (IS). The system suitability test was performed by injecting a mixture of 300 ng/ml MDMA and 500 ng/ml ephedrine (IS) standard solution five times. The repetitive injections resulted in <2% coefficients of variance (CVs). Hence, it met the requirement. DBS samples were prepared using optimised sampling preparation procedures.

A 30 µL aliquot of the spiked blood sample was pipetted onto a dried blood spot (DBS) card and allowed to dry for three hours at room temperature. The dried spot was excised and transferred to a 1.5 ml polypropylene microcentrifuge tube. Exactly 500 µL of methanol with 0.1% HCOOH and 30 µL of ephedrine hydrochloride (500 ng/ml) were added for extraction. The mixture was vortexed at 2000 rpm for 30 seconds, sonicated for 10 minutes, and centrifuged at 10000 rpm for 10 minutes. After evaporating 400 µL of the supernatant using nitrogen at 40°C for 30 minutes, the dried extract was reconstituted in 100 µL of 5 mM NH_4HCOO with 0.1% HCOOH. The clear supernatant was pipetted into an autosampler vial, from which 5 µL was injected into the UPLC-QToF system. The lower limit of quantification (LLOQ) was measured in five replicates. A LLOQ concentration of 20 ng/ml was obtained with a percentage difference of 8.69% to 18.71% and a CV of 3.97%.

The calibration curve consisted of blank samples (without analyte and internal standard), zero samples (without analyte but with internal standard), and non-zero samples (with analyte and internal standard) of 20, 30, 50, 100, 200, 300, and 500 ng/ml. The result was evaluated by observing the linearity, %diff ±20% for LLOQ, and %diff ±15% for non-LLOQ concentration. The calibration curve results met the requirements with %diff ranging between 1.18% and 12.94%. The calibration curve yields a linear regression equation $y = 0.0046x + 0.1168$ with $r = 0.9988$, where x is the MDMA (ng/ml) concentration and y is the peak area ratio (PAR) between MDMA and ephedrine (IS). The calibration curve was made every day before analysis. This

prevented bias caused by measurement errors due to changes in UPLC-QToF conditions and response between days. The mass spectra of MDMA and ephedrine are shown in Figure 1.

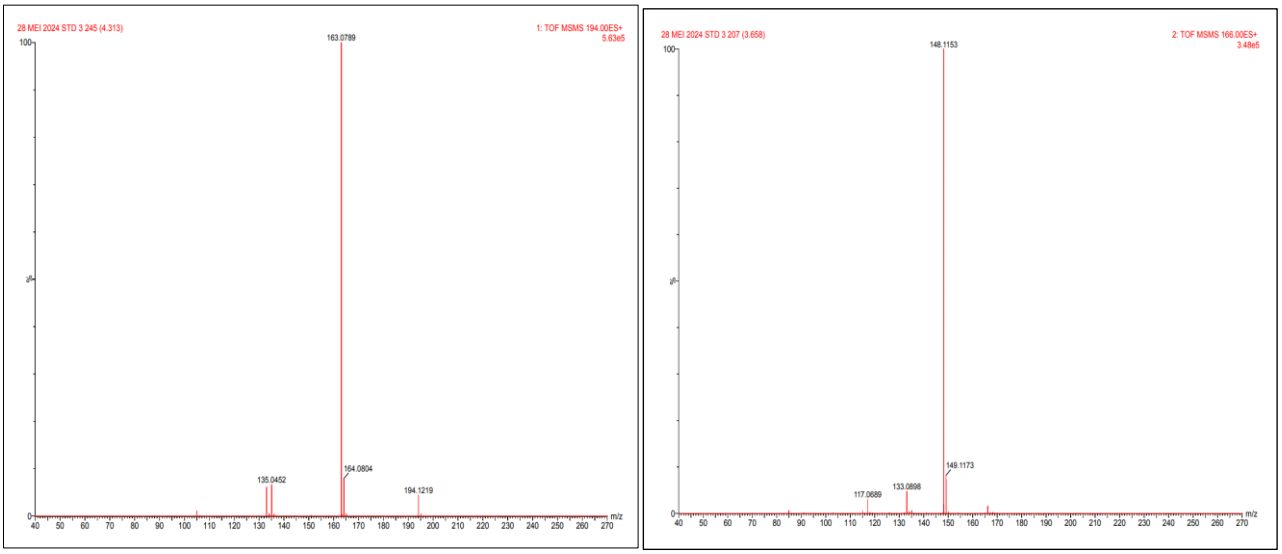

Figure 1: Mass spectrum of MDMA (left) and ephedrine (right)

Following the requirements, a minimum of three replicas of the calibration curve inter-day should be reported to evaluate the precision between curves based on the %CV value against the measured concentration obtained. The linear regression equations obtained from the three replicas were y = 0.0049x + 0.1040 with r = 0.9994; y = 0.0047x + 0.0877 with r = 0.9992; and y = 0.0049x + 0.0924 with r = 0.9994, respectively. The %CV value for LLOQ concentration was 11.87%, while the non-LLOQ concentration ranged from 1.00% to 10.28%. The three replicas met the requirements, with %CV ±20% for LLOQ and ±15% for non-LLOQ.

DBS samples of MDMA in LLOQ quantities and DBS blank samples from six different blood sources were used to assess selectivity. A selectivity test was conducted to determine whether blood extract interference was present. The %interference should be ≤ 20% for LLOQ and ≤ 5% for IS. According to the results, the %interference for MDMA and ephedrine (IS) were 18.90% to 19.62% and 0.37% to 0.77%, respectively. The outcomes satisfied the requirements.

The LLOQ, LQC (Low-Quality Control), MQC (Middle-Quality Control), and HQC (High-Quality Control) %diff and %CV on intra and inter-assays were compared to assess the accuracy and precision. Both assays were carried out with five repetitions. Inter-assays are carried out in three different runs for at least two days. The accuracy of intra and inter-assay was estimated by %diff value ranging from -15.53% to 17.68% for LLOQ and -5.43% to 14.78% for non-LLOQ. The precision of intra- and inter-assay was estimated by a %CV value of 9.93% for LLOQ and a range of 4.18% to 6.73% for non-LLOQ. The recovery was consistent with the average %recovery of 68.32% for MDMA and 7.02% for ephedrine (IS). The %CV values obtained were 2.09% for MDMA and 0.84% for ephedrine (IS).

The DBS blank sample and MDMA at ULOQ concentration (500 ng/ml) were used for the carryover test. The objective of the test was to detect any interference or residual sample in the blank following the injection of a high concentration. The blank sample was injected after the ULOQ injection. The test was done in five replicates. The interference should be ≤20% for MDMA and ≤5% for IS. Based on the result, the %interferences were 19.36% -19.70% for MDMA and 0.28% -0.35 % for ephedrine (IS). The results met the requirements.

Dilution integrity was assessed to investigate whether dilutions affect accuracy and precision. The test was performed using 2 x HQC (800 ng/ml), HQC (400 ng/ml), and ½ HQC (200 ng/ml) with 5 replications. The %diff and %CV values must be ±15%. Based on the result, dilutions did not affect accuracy and precision, with %diff ranging from 4.17% to 14.65% and %CV ranging from 1.12% to 3.01%.

The matrix effect must be assessed because mass spectrometry is used for analysis. The aim is to investigate whether different matrices affect accuracy and precision. The test used six matrices with LQC and

HQC concentrations and five replications. The %diff and %CV values must be ±15%. Based on the results, different matrices did not affect accuracy and precision, with %diff ranging from -11.45 % to 13.80% and %CV ranging from 0.55% to 6.60%.

Reinjection reproducibility was examined to investigate whether reinjections affect accuracy and precision. The test was performed using LQC, MQC, and HQC concentrations with five replications. The %diff and %CV values must be ±15%. Based on the result, reinjections did not affect accuracy and precision, with %diff ranging from 7.30% to 14.34% and %CV ranging from 1.03% to 2.63%.

The short and long-term stability tests were performed on the stock solutions (1 µg/ml MDMA and 1 µg/ml ephedrine), DBS samples (LQC and HQC), and processed samples (LQC and HQC) inside the autosampler (temperature set at 15°C) with three replications each. Based on the results, the stock solutions of MDMA and ephedrine were stable for a minimum of 24 hours at 25°C and a minimum of 14 days at -20°C. The DBS samples were stable for a minimum of 24 hours at 25°C and a minimum of 14 days at -20°C. Lastly, the processed samples were stable for a minimum of 24 hours at 15°C in an autosampler. The chromatograms are shown in Figure 2.

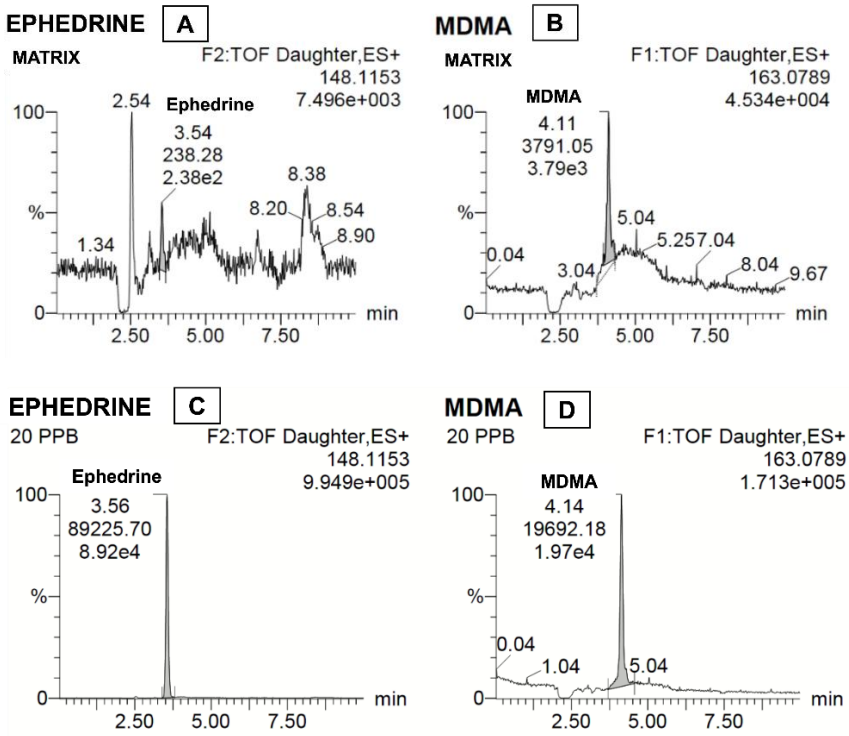

Figure 2: Chromatograms obtained: (A) ephedrine from blank blood extract; (B) MDMA from blank blood extract; (C) ephedrine from LLOQ sample extract; and (D) MDMA from LLOQ sample extract

Discussion

The ESI+ mode was chosen because the analyte and IS were basic compounds with pKa of 9.9 and 9.6, respectively (Moffat *et al*., 2011; National Centre for Biotechnology Information, 2023). In ESI+ mode, the analyte and IS molecules undergo protonation into precursor ions $(M+H)^+$ and are fragmented into fragment ions (Kafeenah *et al*., 2019). The polar mobile phase used in this study was 5 mM NH_4HCOO with 0.1% HCOOH at pH 3. With an acidic compound in the mobile phase, MDMA and ephedrine (IS) would be protonated with an H^+ ion donor mechanism to the nitrogen atom in their molecules. Using NH_4HCOO salt would create a buffer system in the mobile phase so that the pH of the mobile phase would be maintained at three. The environmental setting at pH 3 was intended to ensure that all MDMA and ephedrine were protonated by hydrogen ions from HCOOH. The slowest flow rate and the highest column temperature were used to minimise column back pressure (Li, 1999).

Based on the optimisation results, the variations that resulted in the largest area were all chosen. Blood volume was proportional to the area; thus, a 30 µL blood volume was determined. Three hours of drying

time was optimum to ensure blood diffusion to the DBS card and prevent the risk of degradation during drying. Methanol in 0.1% HCOOH solvent resulted in the largest area because of the acid in it. The analyte and IS were protonated and changed the neutral molecular form into their salt form.

MDMA is an amphetamine base that tends to be volatile but can be trapped in a small amount of acid in its salt, where this change can prevent the loss of analyte and internal standard during evaporation under nitrogen (Thessalonikeos *et al*., 2009). The vortex was intended to mix the solutions inside the tube; the longer the vortex time, the more droplets would be released from the tube during it. Therefore, the shortest vortex time was chosen. The longer the sonication time, the more protein was broken down in the extraction process. However, a longer sonication time would cause an increase in the temperature of the water medium, which puts the analyte at risk of degradation (Wenholz *et al*., 2016). Therefore, ten minutes of sonication time was chosen.

Centrifugation time affects the precipitation process of proteins and impurities in the sample solution; the longer the centrifugation time is, the better the precipitation will be, thus minimising the presence of contaminants. Therefore, ten minutes of sonication time was chosen.

In this study, the development of the MDMA analysis method in DBS was not intended for bioequivalence testing or pharmacokinetic studies but rather to identify and quantify the presence of MDMA compounds to prove drug abuse cases. Therefore, the determination of the LLOQ value was carried out to determine the lowest level of MDMA that could be detected. In a previous study by Pope *et al*. (2021), UPLC-QToF with PRM acquisition mode was used, and a limit of identification (LOI) value of 25 ng/ml was obtained. In this study, the LLOQ value obtained, 20 ng/ml, is smaller than the LOI value obtained in the previous study, although the same instrument was used. Thus, the sensitivity of this method is better.

Conclusion

The MDMA analysis method in DBS using UPLC-QToF met all full validation requirements for selectivity, LLOQ, calibration curve, accuracy, recovery, precision, carryover, matrix effect, dilution integrity, reinjection reproducibility, and stability set by the US FDA (2022). It has demonstrated superiority over previous research with better sensitivity and a more efficient sample preparation method. It could also simplify the sampling method during police investigations of drug abuse and ensure the authenticity of the evidence.

Acknowledgement

This research was supported and funded by the Faculty of Pharmacy, Universitas Indonesia (Grant No. PKS-18/UN.2/F15.D/HKP.05.00/2024) and the Centre for Forensic Laboratory, Criminal Investigation Agency, Indonesian National Police.

References

Allen, D. R., & McWhinney, B. C. (2019). Quadrupole time-of-flight mass spectrometry: A paradigm shift IN toxicology screening applications. *Clinical Biochemist Reviews,* **40**(3), 135–146. https://doi.org/10.33176/AACB-19-00023

Denniff, P., & Spooner, N. (2014). Volumetric absorptive microsampling: A dried sample collection technique for quantitative bioanalysis. *Analytical Chemistry,* **86**(16), 8489–8495. https://doi.org/10.1021/ac5022562

Epshtein, N.A. (2020). System suitability requirements for liquid chromatography methods: Controlled parameters and their recommended values (Review). *Pharmaceutical Chemistry Journal*, **54**, 518–525. https://doi.org/10.1007/s11094-020-02231-w

Gerostamoulos, D., & Schumann, J. (2023). Blood analysis for traditional drugs of abuse. *Encyclopedia of Forensic Sciences* (1, 356–364).

Harahap, Y., Irawan, H., & Kuswardani. (2020). Development and validation of analytical method of 3, 4-methylenedioxy-n-ethylamphetamine in dried blood spot using gas chromatography-mass spectrometry. *International Journal of Applied Pharmaceutics,* **12**(4), 94–99. https://doi.org/10.22159/ijap.2020v12i4.32304

Kafeenah, H. I. S., Osman, R., & Bakar, N. K. A. (2019). Effect of mobile phase pH on the electrospray Ionization efficiency and qualitative analysis of pharmaceuticals in ESI+LC-MS/MS. *Journal of Chromatographic Science,* **57**(9), 847–854. https://doi.org/10.1093/chromsci/bmz061

Kyriakou, C., Marchei, E., Scaravelli, G., García-Algar, O., Supervía, A., & Graziano, S. (2016). Identification and quantification of psychoactive drugs in whole blood using dried blood spot (DBS) by ultra-performance liquid chromatography-tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis,* **128**, 53–60. https://doi.org/10.1016/j.jpba.2016.05.011

Lee, S., Shim, W. S., Yoo, H., Choi, S., Yoon, J., Lee, K. Y., Chung, E. K., Lee, B. C., Yim, S. V., Kim, B. H., & Lee, K. T. (2021). A pharmacokinetic study of ephedrine and pseudoephedrine after oral administration of ojeok-san by validated LC-MS/MS method in human plasma. *Molecules,* **26**(22). https://doi.org/10.3390/molecules26226991

Li, J. B. (1999). *Effect of temperature on column pressure, peak retention time and peak shape*. https://www.waters.com/webassets/cms/library/docs/watersamd30.pdf

Maggadani, B. P., Harahap, Y., Harmita, Haryono, S. J., & Untu, C. W. P. (2021). Analysis of tamoxifen and its metabolites in dried blood spot and volumetric absorptive microsampling: Comparison and clinical application. *Heliyon,* ***7***(6). https://doi.org/10.1016/j.heliyon.2021.e07275

Moffat, A. C., Osselton, M. D., & Widdop, B. (2011). *Clarke's Analysis of drugs and poisons in pharmaceuticals, body fluids and postmortem material (4th ed.)*. Pharmaceutical Press.

National Centre for Biotechnology Information. (2023). *PubChem compound summary for CID 1615, 3,4-methylenedioxymethamphetamine*. https://pubchem.ncbi.nlm.nih.gov/compound/3_4-Methylenedioxymethamphetamine

Odoardi, S., Anzillotti, L., & Strano-Rossi, S. (2014). Simplifying sample pretreatment: Application of dried blood spot (DBS) method to blood samples, including postmortem, for UHPLC-MS/MS analysis of drugs of abuse. *Forensic Science International,* **243**, 61–67. https://doi.org/10.1016/j.forsciint.2014.04.015

Pope, J. D., Black, M. J., Drummer, O. H., & Schneider, H. G. (2021). Urine toxicology screening by liquid chromatography time-of-flight mass spectrometry in a quaternary hospital setting. *Clinical Biochemistry,* **95**, 66–72. https://doi.org/10.1016/j.clinbiochem.2021.05.004

Sadler Simões, S., Castañera Ajenjo, A., & Dias, M. J. (2018). Dried blood spots combined to an UPLC–MS/MS method for the simultaneous determination of drugs of abuse in forensic toxicology. *Journal of Pharmaceutical and Biomedical Analysis,* **147**, 634–644. https://doi.org/10.1016/j.jpba.2017.02.046

The National Narcotics Agency. (2023). *Indonesia Drug Report 2023*. Jakarta: Badan Narkotika Nasional.

Thessalonikeos, E., Tsoukali, H., Spagou, K., Vlachou, M., Pouliopoulos, A., & Raikos, N. (2009). Development of a liquid-liquid extraction procedure for the analysis of amphetamine in biological specimens by GC-FID. *The Open Forensic Science Journal,* **2**(1), 12–15. https://doi.org/10.2174/1874402800902010012

US Food and Drug Administration. (2022). *M10 bioanalytical method validation and study sample analysis: Guidance for industry*. https://www.fda.gov/media/162903/download

Wenholz, D. S., Luong, S., Philp, M., Forbes, S. L., Stuart, B. H., Drummer, O. H., & Fu, S. (2016). A study to model the post-mortem stability of 4-MMC, MDMA and BZP in putrefying remains. *Forensic Science International,* **265**, 54–60. https://doi.org/10.1016/j.forsciint.2016.01.006