Identification of Ketamine in Urines of Nightclubbers Using Paper Spray High-Resolution Mass Spectrometry
การพิสูจน์เอกลักษณ์สารคีตามีนในปัสสาวะของนักเที่ยวกลางคืน ด้วยวิธีเปเปอร์สเปรย์ ไฮ-เรโซรูชัน แมสสเปคโตรเมทรี

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Abstract
Ketamine abuse among nightclubbers is becoming popular among Thai teenagers. Drug tests is required to be performed with the standardized and rapid method to identify the compounds of abuse. Solid phase extraction liquid chromatography-mass spectrometry/mass spectrometry (SPE LC-MS/MS) is the standard method but possesses disadvantage of time consuming. Paper spray high-resolution mass spectrometry (PS-HRMS) is a novel, rapid identification method that does not need the process of sample preparation. This study aims to explore ketamine abuse pattern in nightclubbers and assesses the possibility of applying PS-HRMS to identify ketamine in the suspect's urine sample comparing with the standard method, SPE LC-MS/MS. A total of 149 ketamine positive urine samples of nightclubbers were examined for the pattern of ketamine abuse using SPE LC-MS/MS while 20 randomized urine samples were analyzed for ketamine concentration using PS-HRMS and SPE LC-MS/MS. The results demonstrated pattern of ketamine use that 51.68% of the cases using only ketamine, 38.93% using ketamine and MDMA while the remaining minor of the cases using ketamine with others. Comparing concentrations of ketamine in 20 urine samples detected by both methods, it was demonstrated that concentrations of ketamine detected by PS-HRMS were mostly lower than those detected by SPE LC-MS/MS, but PS-HRMS showed good qualitative agreement with SPE LC-MS/MS. The total analysis times between PS-HRMS and SPE LC-MS/MS were 40 and 600 minutes, respectively. Thus, PS-HRMS was appropriated for qualitative rapid determination of ketamine in urine.

Keywords: Ketamine, Paper spray high-resolution mass spectrometry, Solid phase extraction liquid chromatography-mass spectrometry/mass spectrometry

บทคัดย่อ

การเสพสารคีตามีนเริ่มเป็นที่นิยมในหมู่นักเที่ยวกลางคืนวัยรุ่นไทย การตรวจเอกลักษณ์เพื่อให้ทราบชนิดของสารเสพติดจำเป็นต้องใช้วิธีการตรวจที่ได้มาตรฐานและรวดเร็ว วิธีเอสพีอี แอลซี-เอ็มเอส/เอ็มเอส (SPE LC-MS/MS) เป็นวิธีมาตรฐาน แต่มีข้อด้อยที่ต้องใช้เวลาในการตรวจวิเคราะห์นาน วิธีเปเปอร์สเปรย์ ไฮ-เรโซรูชัน แมสสเปคโตรเมทรี (PS-HRMS) เป็นเทคนิคใหม่ในการวิเคราะห์เอกลักษณ์สารได้อย่างรวดเร็วเนื่องจากไม่มีขั้นตอนการเตรียมตัวอย่าง การวิจัยนี้มีวัตถุประสงค์เพื่อสำรวจรูปแบบการใช้คีตามีนในนักเที่ยวกลางคืนและศึกษาความเป็นไปได้ในการประยุกต์ใช้วิธี PS-HRMS ในการพิสูจน์เอกลักษณ์คีตามีนในปัสสาวะผู้ต้องสงสัย เปรียบเทียบกับวิธีมาตรฐาน SPE LC-MS/MS ในการวิจัยได้ทำการสำรวจรูปแบบการใช้คีตามีนในนักเที่ยวกลางคืน โดยใช้ตัวอย่างปัสสาวะจำนวนรวม 149 รายทำวิเคราะห์เอกลักษณ์คีตามีนด้วยวิธี SPE LC-MS/MS และทำการสุ่มตัวอย่างมา 20 รายเพื่อวิเคราะห์ความเข้มข้นของคีตามีนด้วยวิธี PS-HRMS และ SPE LC-MS/MS ผลการทดลองพบว่า รูปแบบการใช้คีตามีนในตัวอย่างที่ศึกษาส่วนใหญ่ใช้คีตามีนอย่างเดียวมีจำนวน 51.68% ใช้คีตามีนร่วมกับ MDMA จำนวน 38.93% ที่เหลือเป็นส่วนน้อยใช้คีตามีนร่วมกับสารเสพติดอื่น เมื่อเปรียบเทียบความเข้มข้นของคีตามีนในปัสสาวะจำนวน 20 ตัวอย่างที่ทำการวิเคราะห์โดยทั้งสองวิธี พบว่ามีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ โดยความเข้มข้นของคีตามีนที่ตรวจโดยวิธี PS-HRMS ส่วนใหญ่มีค่าต่ำกว่าวิธี SPE LC-MS/MS แต่ผลการวิเคราะห์เชิงเอกลักษณ์ของวิธี PS-HRMS ได้ผลบวกสอดคล้องกับวิธี SPE LC-MS/MS ระยะเวลาโดยรวมที่ใช้ในการวิเคราะห์ตัวอย่างทั้งหมด 20 ตัวอย่างด้วย วิธี PS-HRMS และวิธี SPE LC-MS/MS เท่ากับ 40 นาที และ 600 นาที ตามลำดับ ดังนั้นวิธี PS-HRMS จึงเหมาะสำหรับการวิเคราะห์เอกลักษณ์คีตามีนในปัสสาวะที่มีความถูกต้องและรวดเร็ว

คำสำคัญ: คีตามีน เปเปอร์สเปรย์ ไฮ-เรโซรูชัน แมสสเปคโตรเมทรี โซลิดเฟสเอกแทกชัน ลิควิดโครมาโตร กราฟี-แมสสเปคโตรเมทรี/แมสสเปคโตรเมทรี

Introduction

Teenagers and other nightclubbers use several drugs of abuse called 'club drugs' for entertainment. The United Stated National Institute on Drug Abuse (NIDA) defines club drugs as drugs or compounds used at bars, nightclubs, or parties, including Gamma-hydroxybutyrate --GHB, flunitrazepam, ketamine, ecstasy (3-4 Methylenedioxy-methamphetamine, MDMA), methamphetamine and LSD, etc. (Kelly, Parsons & Wells, 2006) However, different regions and cultures may define differently, such as Thailand, club drugs include ketamine and ecstasy and sometimes include methamphetamine crystal but not GHB, flunitrazepam and LSD like in the US.

Thai nightclubbers prefer to take ketamine by snorting or dissolve in alcoholic beverages, while ecstasy is consumed orally as tablets (Areesantichai, Perngparn & Boonbundarlchai, 2020). Methamphetamine is usually used by burning with lighters and smoking, which is not convenient to take in nightclubs. Because ecstasy acts slowly, and the effect loses slowly too. The nightclubbers often take it before or when entering the nightclub or take it at the beginning of parties. When dancing for a while, they will continue to take ketamine to create a more psychedelic mood. Ketamine has a fast onset of action but short duration. It is inactive within less than two hours after use. After that, ketamine abuser will feel like the spirit is snatched from the body.

The body cannot voluntarily move. This is called dissociation effect and the hallucination such as a dreamlike image or the feeling like floating in the air (Sassano-Higgins, Baron, Juarez, Esmaili & Gold, 2016).

In the past two years, most Thai nightclubbers arrested in many pubs, nightclubs or parties in Bangkok have been detected with ketamine positive using drug-abuse screening test kits. Usually, drug abuse identification in nightclubbers need to be performed as soon as possible and require quick results to rule out the negative drug abuse person from the mass sample size of arrested suspects. Therefore, police should choose the standardized and fastest method to detect and confirm the type of drug in biological samples of nightclubbers. Solid phase extraction liquid chromatography-mass spectrometry/mass spectrometry (SPE LC-MS/MS) is recently the most suitable method as it is the fastest method to confirm the type of drugs. It is convenient for the analyzers because there is no need to do manual extraction with solid phase extraction or liquid-liquid extraction before analyzing with LC-MS/MS. However, this method still takes approximately 30 minutes per one sample determination. Paper spray high-resolution mass spectrometry (PS-HRMS) is a new rapid identification method that does not require the sample preparation process thus take a shorter run period (about 2 minutes per sample). The first report of the PS-HRMS method (Wang, Lui, Cooks & Ouyang, 2010), explained that PS-HRMS is a usual ambient ionization method that is potentially versatile direct analysis method for raw bio-fluid samples with significant quantitative power. Nowadays, many studies have published various modifications, techniques, and potential applications that demonstrate the rapid, quantitative ability of PS-HRMS through targeted analysis of multiple molecules such as narcotic and therapeutic drugs in biological specimens (McKenna, Jett, Shanks & Manicke, 2018). Among the compounds studied using PS-HRMS, few studies focus on developing this method to determine ketamine in urine but in postmortem blood (McKenna et al., 2018). In this study, we used urine samples which were usually collected readily from the suspects at the sites of arrested. This is because ketamine is excreted mostly in urine in form of unchanged (2%) and mostly in the form of metabolites. Conjugated hydroxylated derivatives of ketamine (80%) followed by dehydronorketamine (16%) are the most prevalent metabolites detected in urine (Zanos et al., 2018).

Objective

This study aims to explore ketamine abuse pattern in nightclubbers and assesses the possibility of applying PS-HRMS to identify ketamine in the suspect's urine sample comparing with the standard method, SPE LC-MS/MS.

Literature review

Paper spray mass spectrometry (PS-MS) has been introduced since 2010. It is a rapid method performing direct analysis of samples (such as blood or urine) by spotting on to the paper and detecting by MS. Several groups of researchers have demonstrated the applications of paper spray MS to examine various therapeutic drugs and drugs of abuse.

Kennedy et al. (2016) described the use of paper spray MS to identify synthetic cannabinoids and cathinone, two common classes of Novel

Psychoactive Substances--NPS, that mimic the effects of tetrahydrocannabinol but often elude detection by current drug screening techniques. These two classes of NPS have been detected in many herbal incense products and powdered bath salts. Their structures are readily modified, without loss of physiological activity, resulting in new NPS-type compounds that evade regulation as controlled substances. The combination of paper spray and high resolution mass spectrometry provides a powerful and simple tool for identification of new substances, without requiring reference standards.

McKenna et al (2018) developed PS-HRMS/MS for drug screening in 30 postmortem blood compared with the results using LC–MS/MS. The results demonstrated that PS-HRMS/MS showed good qualitative agreement with LC–MS/MS; the true positive rate of PS-HR MS/MS was 92%, and the true negative rate was over 98%. The quantitative results between the two methods were also acceptable with a Pearson's correlation coefficient of 0.996.

Vandergrift, Hessels, Palaty, Krogh & Gill (2018) presented the use of PS-MS for direct measurement of fentanyl and norfentanyl in urine or analgesic slurries that mimic street drug preparation. PS-MS was compared with LC-MS for the analyses of real urine samples with satisfactory results. PS-MS shows potential as a sensitive and selective direct measurement strategy for use in fentanyl harm reduction strategies.

Huang et al. (2021) demonstrated the use of PS-MS to distinguish THC and CBD and to quantitate the ratio of THC/CBD in the commercial CBD products. Their developed method was further validated by the analysis of 10 commercial oils. Based on the determined relative concentration ratios of THC/CBD and the declared CBD concentration, 6 out of 10 CBD oils appear to contain more THC than the Dutch legal limit of 0.05%.

Conceptual Framework

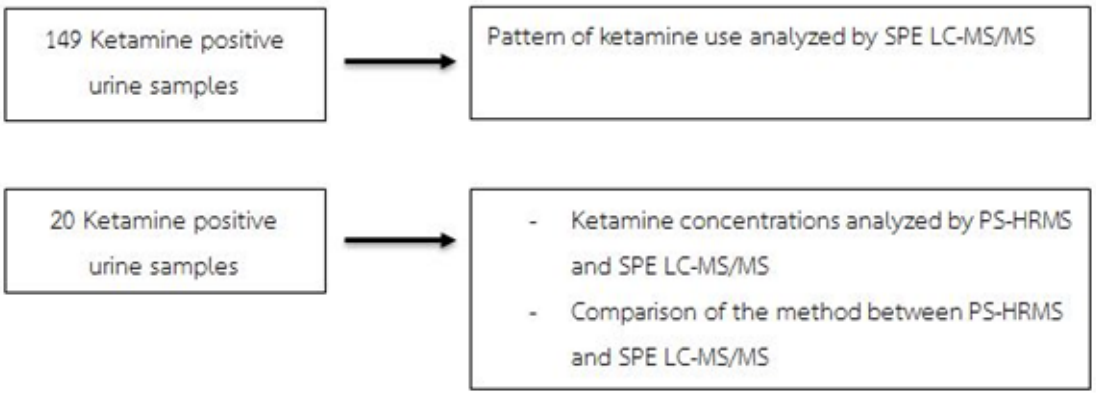

Figure 1 Conceptual Framework

Methodology

Population and Sample:

Urine samples, collected from nightclubbers suspected of using illicit drugs, were sent from various police stations in Bangkok during 2020-2021 to the Institute of Forensic Medicine, Police General Hospital to identify for abuse drugs. The total numbers of 149 samples that were ketamine positive were used to assess the pattern of ketamine used. Twenty ketamine positive urine samples were randomized to determine the concentration of ketamine by both PS-HRMS and SPE LC-MS/MS.

Research Tools:

PS-HRMS including PS (Prosolia Velox 360TM) and HRMS (Orbitrap Thermo Scientific™ Q Exactive Focus™) were from Thermo Fisher ScientificTM, USA. SPE LC-MS/MS including SPE (HyperSep Retain PEP), LC (Ultimate 3000TM) and MS (TSQ QuantivaTM) were from Thermo Fisher ScientificTM, USA. The standard solution of ketamine and other drugs of abuse including amphetamine, methamphetamine, MDMA, cocaine and mitragynine were provided from the Department of Medical Sciences, Ministry of Public Health.

Procedure and Data Collection:

Identification and quantitation of ketamine and other illicit drugs using SPE LC-MS/MS were performed according to the standard method as described in Wang et al (2021).

Quantitation of ketamine in urine using PS-HRMS was briefly described as following: Trimipramine (used as internal standard) was added to the urine sample at 1000 ng/mL. Ten microlitre of the urine was then spotted directly onto a Velox Sample Cartridge. The PS solvent used to extract analyte from the dry urine on the paper of a Velox Sample Cartridge was acetonitrile: water: 10 M acetic acid (90: 10: 0.01). Operated Orbitrap MS was in full-scan data-dependent MS2 mode with high-resolution and full-scan data at a resolution of 70000 was collected and then triggered MS2 spectra at a resolution of 35000 for compounds entered in the inclusion list. Data were acquired with TraceFinder™ software, version 3.2 and analyzed with Thermo Scientific™ ToxFinder™ software, version 1.0. ToxFinder™ software identified compounds based on the exact mass of precursor, isotopic pattern and MS2 spectra.

To determine the Limit of Detection--LOD of the method: The standard solution of ketamine and five drugs were spiked into pooled blank urine at the concentrations of 1, 5, 10, 25, 50, 100, 250, 500 and 1000 ng/mL. Two types of LOD were evaluated using the criteria of McKenna et al. (2018). LOD was based on a chronogram peak area above a specified threshold. $LOD_{confirmed}$ was based on (1) a chronogram peak area above a specified threshold and (2) Isotopic pattern comparison mass error NMT±five ppm and matching scores>80% or (3) Fragment ions matching mass error NMT±five ppm and minimum of fragments needed ≥ 2. LOD_{exact} was based on all 3 criteria of (1), (2) and (3) mentioned above.

Statistical Analysis:

Difference of ketamine concentration determined by PS-HRMS, and SPE LC-MS/MS was analyzed by Wilcoxon matched pairs signed-ranks test using SPSS version 17 at a significant level of $p<.05$.

Result

The LOD, $LOD_{confirmed}$ and LOD_{exact} of PS-HRMS for ketamine detection and other five drugs of abuse were presented in Table 1. Using the criteria of McKenna et al (2018), LOD of PS-HRMS for ketamine detection was 25 ng/mL with $LOD_{confirmed}$ of 50 ng/mL and LOD_{exact} that passed all 3 criteria of 250 ng/mL.

A total of 149 ketamine positive urine samples of nightclubbers included 87 males (58.39%) and 62 females (41.61%), with an average age of 24.76±4.06 years (range 20-44). Most of the cases (51.68%) used only ketamine, 38.93% used ketamine and MDMA while the remaining minor of the cases used ketamine with others (Table 2).

Twenty randomized urine samples were analyzed for ketamine concentrations using PS-HRMS and SPE LC-MS/MS. The analytical results were shown in Figure 2, and the statistical test for the difference of ketamine concentration between both methods was shown in Table 3. It was shown that ketamine concentrations examined by PS-HRMS were mostly lower than those examined by SPE LC-MS/MS despite showing consistently same positive ketamine results. (Figure. 2). As comparing the differences of ketamine concentration in urine detected by both methods using Wilcoxon matched pairs signed-ranks test, statistically significant difference was seen (Table 3). The total analysis times of these 20 samples between PS-HRMS and SPE LC-MS/MS methods were 40 and 600 minutes, respectively.

Discussions

In this study, we found that ketamine abuse pattern in Thai nightclubbers was shown to primarily use ketamine alone (51.68%) or use ketamine in combination with MDMA (38.93%). Since ketamine is classified as psychotropic substance according to the Psychotropic Substances Act. 2518 B.E. (1975 AD) while MDMA and other amphetamines are classified as narcotic drugs according to the Narcotics Act. 2522 B.E. (1979 AD), ketamine abusers receive less penalty than MDMA abusers. Therefore, the suspects who are found positive ketamine during drug screening test, should be tested for other narcotic drugs as well.

In this study, PS-HRMS possessed LOD and $LOD_{confirmed}$ for ketamine of 25 ng/mL and 50 ng/mL, respectively. Moreover, the LOD_{exact} was 250 ng/mL, all three values are below the positive threshold of 1,000 ng/mL of the strip test and the cut-off value of ketamine (1 µg/ml) in the Official Announcement of the Narcotic Control Board (2020). McKenna et al. (2018) developed PS-HR MS/MS for drug screening assays using a quadrupole-orbitrap mass spectrometer, semi-quantitatively screened over 130 drugs and drug metabolites at sub-toxic concentrations. They reported the LOD of ketamine in post-mortem blood of 100 ng/mL. Therefore, PS-HR MS/MS used

in our study to determine ketamine concentration in urine possessed the satisfactory LOD.

This study determined ketamine concentrations using PS-HRMS compared to SPE LC-MS/MS. Concentrations of ketamine detected by the PS-HRMS method were mostly lower than those detected by the SPE LC-MS/MS. As comparing the differences of ketamine concentration in urine detected by both methods using Wilcoxon matched pairs signed-ranks test, statistically significant difference was seen. However, PS-HRMS showed good qualitative agreement with SPE LC-MS/MS. A group of researchers have suggested the important impacts that affect performance of this method such as the appropriate solvent and the mode of applying the solvent (Ren et al., 2013). In addition, another group of researchers reported the use of Modified-PS by adding SPME (solid phase micro extraction) fiber extraction techniques before importing PS-HRMS. They spiked urine samples with three substances including ketamine, p-chloroamphetamine and MDMA and then analyzed by that modified method followed by comparing the results with those using the typical methods, PS-HRMS. The results showed that the limit of detection can be dramatically improved (Wang, Lai, Liou, Chen & Lin, 2015).

The advantage of the PS-HRMS method is that it does not require sample preparation and chromatographic process. Therefore, this method reduces analysis time from hour to only a few minutes. Since PS-HRMS quantitatively and directly determine the substance using mass spectrometry without the chromatographic process. Therefore, the detector unit of the equipment requires high-efficient property. The paper-spray machine needs to be connected to the mass spectrometry range from the triple quadrupole mass spectrometer to the high-resolution mass spectrometry (HRMS) to reduce the weaknesses of the absence of chromatographic processes. In this research, the high-resolution MS (Orbitrap) was used. According to this study, the analysis time of examining ketamine in urines of 20 cases took only 40 minutes for the PS-HRMS method but 600 minutes as using the SPE-LC-MS/MS method, which is normally the fast chromatography method. In our study, we found that PS-HRMS method could save time 15 folds as compared to the standard method (SPE-LC-MS/MS).

Taken together, PS-HRMS showed good qualitative agreement with SPE LC-MS/MS accompanying with its rapid analysis. Thus, PS-HRMS was appropriated for qualitative rapid determination of ketamine in urine.

In conclusion, the pattern of ketamine abuse in Thai nightclubbers was found to prefer using ketamine alone or using ketamine with MDMA. LOD and LODconfirmed of PS-HRMS for ketamine determination in urines were 25 ng/mL and 50 ng/mL, respectively. Concentrations of ketamine detected by PS-HRMS were mostly lower than those detected by the standard method, SPE-LC-MS/MS but PS-HRMS showed good qualitative agreement with SPE LC-MS/MS. The advantage of using PS-HRMS was that the analysis time of PS-HRMS was much less than that of the standard method.

Table 1

LOD, $LOD_{confirmed}$ and LOD_{exact} of PS-HRMS for ketamine detection and other five standard samples

Drug abuse	Concentration		
	LOD (ng/mL)*	$LOD_{confirmed}$(ng/mL)*	LOD_{exact}(ng/mL)*
Ketamine	25	50	250
Amphetamine	1	50	100
Methamphetamine	1	250	500
MDMA	1	50	100
Cocaine	1	50	100
Mitragynine	1	10	25

*Data obtained from 3 repetitions of the test.

Table 2

Ketamine abuse pattern shown in 149 ketamine positive urine samples determined by SPE LC-MS/MS

Drug abuse	Gender		Frequency	Relative frequency (%)
	male	female		
Ketamine only	48	29	77	51.68
Ketamine and MDMA	27	31	58	38.93
Ketamine, methamphetamine and MDMA	3	1	4	2.68
Ketamine, MDMA and mitragynine	3	-	3	2.01
Ketamine and methamphetamine	2	1	3	2.01
Ketamine and mitragynine	4	-	4	2.68
Total	**87**	**62**	**149**	**100.0**

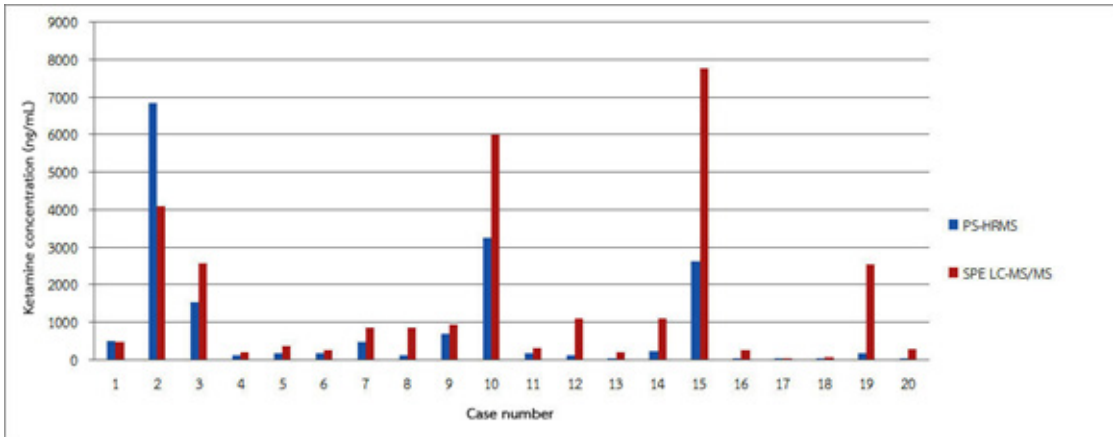

Figure 2 Ketamine concentrations in 20 urine samples determined by PS-HRMS and SPE LC-MS/MS

Table 3

Statistical analysis of ketamine concentrations (ng/mL) in 20 urine samples determined by PS-HRMS and SPE LC-MS/MS methods

Method	Ranks	N	Mean Rank	Sum of Ranks	Z	*p*
PS-HRMS-SPE LC-MS/MS	Negative Ranks (PS-HRMS < SPE-LCMS/MS)	18	10.44	188.00	-3.099	.002
	Positive Ranks (PS-HRMS > SPE-LCMS/MS)	2	11.00	22.00		

Recommendation

In this study, ketamine concentrations determined by PS-HRMS was mostly lower than those detected by the standard method, SPE-LC-MS/MS. As suggested in the study of Ren et al. (2013), improper PS solvent is the important impact of the detection efficiency. Further studies should be performed to find the more appropriate PS solvent solutions for extracting/eluting ketamine from biomaterial. In addition, more sample size should be considered to increase the confidence of using PS-HRMS for qualitative and quantitative applications for ketamine determination in the future.

References

Areesantichai, C., Perngparn, U., & Boonbundarlchai, R. (2020). Ketamine use among nightlife people. *ONCB Journal, 36*(1), 46-53. (in Thai)

Huang, S., Claassen, F. W., van Beek, T. A., Chen, B., Zeng, J., Zuilhof, H., & Salentijn, G. I. (2021). Rapid distinction and semiquantitative analysis of THC and CBD by silver-impregnated paper spray mass spectrometry. *Analytical chemistry, 93*(8), 3794–3802. https://doi.org/10.1021/acs.analchem.0c04270

Kelly, B. C., Parsons, J. T., & Wells, B. E. (2006). Prevalence and predictors of club drug use among club-going young adults in New York city. *Journal of Urban Health: Bulletin of the New York Academy of Medicine, 83*(5), 884–895. https://doi.org/10.1007/s11524-006-9057-2

Kennedy, J., Shanks, K. G., Natta, K. V., Conaway, M. C. P., Wiseman, J. M., Laughlin, B., & Kozak, M. (2016). Rapid screening and identification of novel psychoactive substances using paper spray interfaced to high resolution mass spectrometry. *Clinical Mass Spectrometry, 1*, 3–10. http://dx.doi.org/10.1016/j.clinms.2016.08.003

McKenna, J., Jett, R., Shanks, K., & Manicke, N. E. (2018). Toxicological drug screening using paper spray high-resolution tandem mass spectrometry (HR-MS/MS). *Journal of Analytical Toxicology, 42*(5), 300–310. https://doi.org/10.1093/jat/bky001

Official Announcement of the Narcotic Control Board, (2020, 17 Jul). *The Royal Thai Government Gazette*. Volume. 137 Part 164, pp. 18-22. Retrieved from http://www.ratchakitcha.soc.go.th/DATA/PDF/2563/E/164/T_0018.PDF. (in Thai)

Ren, Y., Wang, H., Liu, J., Zhang, Z., McLuckey, M. N., & Ouyang, Z. (2013). Analysis of biological samples using paper spray mass spectrometry: An investigation of impacts by the substrates, solvents and elution methods. *Chromatographia, 76*(19-20), 1339–1346. https://doi.org/10.1007/s10337-013-2458-y

Sassano-Higgins, S., Baron, D., Juarez, G., Esmaili, N., & Gold, M. (2016). A review of ketamine abuse and diversion. *Depression and Anxiety, 33*(8), 718–727. https://doi.org/10.1002/da.22536

Vandergrift, G. W., Hessels, A. J., Palaty, J., Krogh, E. T., & Gill, C. G. (2018). Paper spray mass spectrometry for the direct, semi-quantitative measurement of fentanyl and norfentanyl in complex matrices. *Clinical biochemistry, 54*, 106–111. https://doi.org/10.1016/j.clinbiochem.2018.02.005

Wang, H., Liu, J., Cooks, R. G., & Ouyang, Z. (2010). Paper spray for direct analysis of complex mixtures using mass spectrometry. *Angewandte Chemie (International ed. in English), 49*(5), 877–880. https://doi.org/10.1002/anie.200906314

Wang, J. S., Lai P. H., Liou Y.W., Chen C. C. & Lin C. H. (2015). The use of a beveled porous-polypropylene hollow fiber for liquid-liquid microextraction in paper spray- mass spectrometry. *Journal of Chromatography Separation Techniques, 6*(7), 1-4. http://dx.doi.org/10.4172/2157-7064.1000311

Wang, J., Qi, L., Hou, C., Zhang, T., Chen, M., Meng, H., Su, M., Xu, H., Hua, Z., Wang, Y., & Di, B. (2021). Automatic analytical approach for the determination of 12 illicit drugs and nicotine metabolites in wastewater using on-line SPE-UHPLC-MS/MS. *Journal of Pharmaceutical Analysis, 11*(6), 739–745. https://doi.org/10.1016/j.jpha.2021.01.002

Zanos, P., Moaddel, R., Morris, P. J., Riggs, L. M., Highland, J. N., Georgiou, P., Pereira, E. F. R., Albuquerque, E. X., Thomas, C. J., Carlos A. Zarate, Jr. C. A., & Todd D. Gould, T. D. (2018). Ketamine and ketamine metabolite pharmacology: Insights into therapeutic mechanisms. *Pharmacological Reviews, 70*, 621-660. https://doi.org/10.1124/pr.117.015198