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Paper spray mass spectrometry: A new rapid confirmation method for methamphetamine

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Abstract

Paper spray mass spectrometry (PS-MS) is amenable for analyzing drugs and other compounds in biological samples. It has provided a rapid, qualitative, and quantitative ambient ionization method for biomolecules analysis to an alternative to traditional sample preparation and chromatography. This research aimed to study the efficiency of postmortem urine methamphetamine (METH) identification using PS-MS (orbitrap) compared with the Online-SPE-LC-MS/MS method. Twenty-one of METH positive urine and four METH negative urine from May 2017-Dec 2019 were randomly sampled and analyzed for METH concentration using both methods. The qualitative results obtained by the PS-MS method founding that twenty-one of METH positive urine cases were passing of three criteria parameters of PS-MS method with the true positive rate equal to 100% and the quantitative results founding that METH concentrations determined by PS-MS method were significantly higher than the results from Online-SPE-LC-MS/MS method (Paired t-Test, P-value < 0.05). PS-MS method can save time 7.5 fold as compared to the Online-SPE-LC-MS/MS method. In conclusion, PS-MS can be used for rapid screening and confirmation of urine METH.

Keywords: Methamphetamine, paper spray mass spectrometry, Orbitrap

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1. Introduction

Methamphetamine (METH) is one of the most commonly used illicit drugs in the world. [1] It is a highly addictive psycho-stimulant that belongs to a class of synthetic drugs called amphetamine-type stimulants (ATSs). [2] A new report (2020) from the United Nations Office on Drugs and Crime (UNODC) warns that the synthetic drug market in East and Southeast Asia continues to expand and diversify. The price of METH has dropped to the lowest level in a decade as the supply has surged [3] then increasing the number of Thai addictions. METH is classified as a penal drug in category-1 of the Narcotics Act, B.E. 2522; the use of METH is still prohibited. Furthermore, Narcotics Addict Rehabilitation Act, B.E. 2545 ordered the METH-addicted must be committed to rehabilitating. With the consequence of both laws, the suspect will be tested for drug use in the body, such as a urine test for METH. Urine METH concentrations greater than 1000 ng/mL are considered to exceed the legal limits. This value is called the cut-off limits, which must be examined confirmation in the next step. Forensic toxicology laboratories typically use a two-step process to detect toxicants in biological samples. The first step is screening, which employs a variety of analytical procedures to detect a broad range of targets such as Immunoassays (IAs), gas chromatography (GC), GC-mass spectrometry (MS), liquid chromatography-MS (LC-MS) or LC-MS/MS [4] are all commonly used methods for drug screening. Positive results during the initial drug screening step are confirmed and quantitated by an independent analytical procedure. LC-MS/MS is the most widely used drug confirmation and quantitation method due to its excellent sensitivity and selectivity. In Thailand, the suspect urine will be tested twice. The first step is screening with the immune-chromatography test kit that has cut-off values according to the law. The second step confirms with Online Solid-phase extraction-liquid chromatography-mass spectrometry/mass chromatography (Online SPE-LC-MS/MS). The Confirmation step is the most time-consuming in the entire identification process [5] because it consists of the procedure for specimen preparation and chromatographic separation. Thus it takes an average of 30 minutes per sample. Paper spray MS (PS-MS) is a novel approach for rapid drug screening and confirmation. The biological sample is spotted onto a paper substrate. Upon application of a spray solvent and electric potential, extraction and ionization occur directly from the paper without any need for additional sample preparation. Then it is spending a shorter run time period (approximately 2 minutes per sample). [6] Due to its simplicity and efficacy, PS-MS has garnered significant interest. Numerous papers have published vari-

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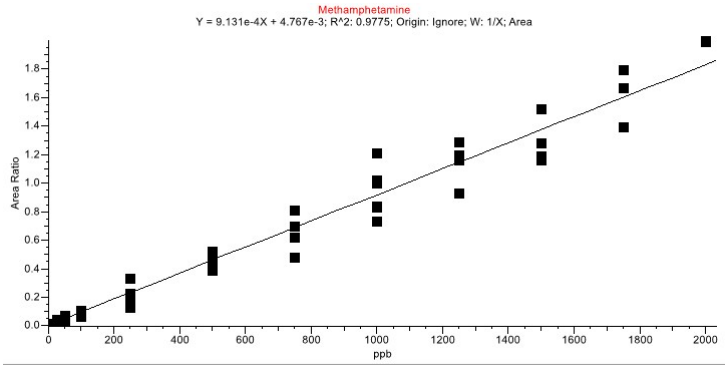

Figure 1: METH calibration curves for PS-MS.

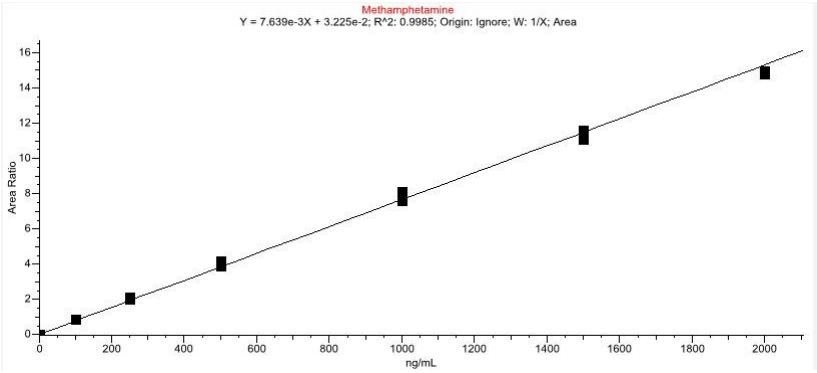

Figure 2: METH calibration curves for Online SPE-LC-MS/MS.

ous modifications, methods, and potential applications that demonstrate the rapid, quantitative power of PS-MS through targeted analysis of various molecules, including illicit drugs, therapeutic drugs, metabolites, lipids, and proteins in biomedical samples.[7 – 9] This research aims to study the efficiency of urine METH identification using PS-MS compare with the Online-SPE-LC-MS/MS method.

2. Materials and Method

Twenty-one of METH positive urine and four of METH negative urine from May 2017-Dec 2019 were randomly sampled from sample discard unit; SDU (collected the samples that cases have ended), the Institute of Forensic Medicine, Police General Hospital. The standard METH solution was obtained from the Department of Medical Sciences, Ministry of Public Health. PS-MS, PS: Prosolia Velox 360, MS: Orbitrap Q Exactive Focus and Online-SPE-LC-MS/MS, LC: Ultimate3000, MS: TSQ Quantiva were from Thermo Fisher Scientific, San Jose, CA, USA.

Trimipramine (1,000 ng/mL) was added to urine samples as an internal standard, and 10 μL of the urine was spotted directly onto a Velox sample cartridge. The PS solvent used for extraction analyte from dry urine in the paper of a Velox sample cartridge was Acetonitrile: water: 10 M acetic acid (90: 10: 0.01). The Orbitrap mass spectrometer was operated in full-scan data-dependent MS^2 Mode. In this mode, high-resolution, full-scan data at a resolution of 70000 were collected, then MS^2 spectra at a resolution of 35000 were triggered for compounds entered in the inclusion list. Data were acquired with TraceFinder? software, version 3.2, and analyzed with ToxFinder? software, version 1.0. This software used to identify compounds based on three parameters (1) a chronogram peak area above a specified threshold (2) Isotopic pattern comparison, mass error NMT ±5 ppm, and matching scores > 80% and (3) Fragment ions matching mass error NMT ±5 ppm and a minimum of fragments needed ≥ 2. Postmortem blank urine were spiked with standard METH at concentrations of 1, 5, 10, 25, 50, 100, 250, 500, 750, 1000, 1250, 1500, 1750 and 2000 ng/mL as a standard solution. Standard curves for PS-MS and Online-SPE-LC-MS/MS were plotted, 25 urine samples were evaluated.

3. Results and Discussion

METH calibration curves (n = 14 standards, four injections per standard) for PS-MS were evaluated in the range 1 - 2000 ng/mL, showing to be linear equation Y = 9.131 e-4x + 4.767 e-3 with r^2 = 0.9775 (Figure 1) and METH calibration curves (n = 7 standards, two injections per standard) for Online SPE-LC-MS/MS was showing to be linear equation Y = 7.639 e-3x + 3.225 e-2 with r^2 = 0.9985 (Figure 2).

Twenty-five randomized urine samples were analyzed for METH concentrations using PS-MS and

Table 1. Differences of METH concentrations from 21 positive urine samples analyzed by PS-MS and Online-SPE-LC-MS/MS method.

Method	n	Mean (ng/mL)	Std. Deviation	Paired Differences		t	P-value
				Mean	Std. Deviation		
PS-MS	21	30517.60	23571.09	17685.39	16363.59	4.953	0.000
Online-SPE-LC-MS/MS	21	12832.22	7844.23				

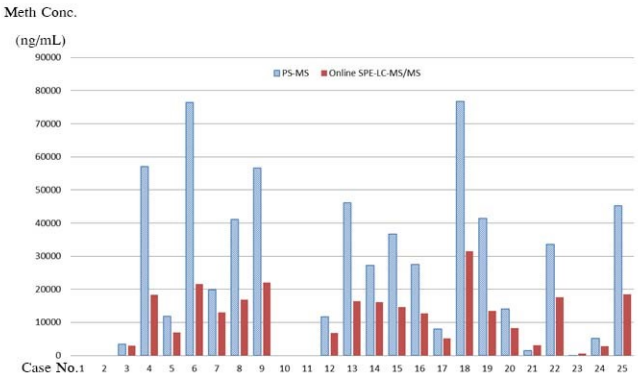

Figure 3: The concentration of METH urine samples from 25 samples by both PS-MS and Online-SPE-LC-MS/MS method.

Online-SPE-LC-MS/MS method. The analysis results were shown in Figure 3.

The qualitative results obtained by the PS-MS method founding that twenty-one METH positive urine cases were passing of three criteria parameters of the PS-MS method with the true positive rate equal to 100%. These results indicate that the method shows good promise as a drug screening method, consistent with the study of McKenna *et al.* [10] using PS- MS (orbitrap) to screen 130 various drugs including METH in blood specimen, the true positive rate was 92.1%. The PS-MS screening needs a high true positive rate because it used high-resolution MS that presents high-quality screening outcomes. If fewer targets were included in the screening panel, the resolution could improve selectivity and sensitivity automatically.

The quantitative results obtained by the PS-MS method were compared to the Online-SPE-LC-MS/MS quantitative confirmations using Passing-Bablok regression (Figure 4). The two methods were highly correlated, with a Spearman rank correlation coefficient (r) of 0.949. The concentrations determined by PS-MS were, on average, higher than the LC-MS/MS values as reflected by the slope value of 2.8998. Although the PS-MS technique was developed as a rapid screening method and the LC-MS/MS method was developed for quantitative confirmation. However, the quantitative performance of PS-MS can be improved by decreasing the number of targets, and by using isotopically labeled internal standards for each analyte. [10] For example, McKenna *et al.* used Methamphetamine-d^{11} as an internal standard for detection METH from postmortem blood.

Differences between METH concentrations detected by both methods were analyzed using the Paired t-Test. It was shown that the METH concentration determined by both methods were the statistically significant difference at the level of P-value < 0.05. (Table 1), consistent with slope value from Passing-Bablok regression indicated that PS solvent in this study (90: 10: 0.01 acetonitrile: water: 10 M acetic acid) more suitable for extract METH from the paper cartridge and released METH in large quantities whereas the Online SPE-LC-MS/MS may cause loss of yield from the extraction process. However, it can be done if use the PS-MS method as a qualitative confirmation method after the initial screening.

However, the total analysis time of PS-MS was much less than that of the Online-SPE-LC-MS/MS method (7.5 folds).

4. Conclusion

PS-MS is amenable for the analysis of drugs and other compounds in biological samples. In this study, the PS-MS was assessed to determine METH in postmortem urine samples compared to the standard Online-SPE-LC-MS/MS method. PS-MS showed good promise as a drug screening method in quantitative propose. METH concentrations analyzed by PS-MS were higher than the LC-MS/MS method. However, the total analysis time of PS-MS was much less than that of the Online-SPE-LC-MS/MS method. In conclusion, PS-MS (orbitrap) can be used for rapid screening and confirmation of urine METH.

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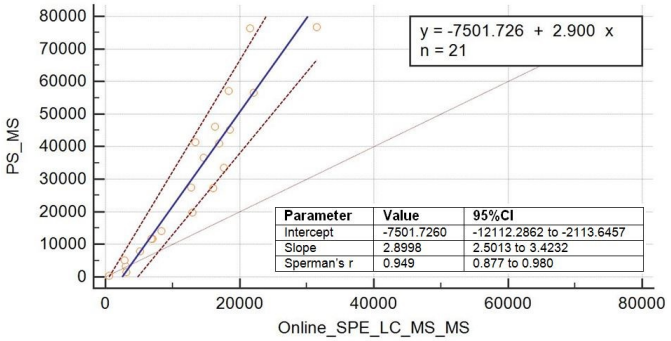

Figure 4: Comparison of METH concentrations from 21 positive urine samples by both PS-MS and Online-SPE-LC-MS/MS method. The Passing-Bablok regression was shown as a solid line. The ± 95%CI was shown as a dashed line.

References

[1] I. N. Krasnova, J. L. Cadet, Methamphetamine toxicity and messengers of death, Brain Research Reviews 60(2) (2009) 379 – 407.

[2] C. Chomchai, S. Chomchai, Global patterns of methamphetamine use, Current Opinion in Psychiatry 28(4) (2015) 269 – 274.

[3] UNODC, United Nations Office on Drug and Crime (UNODC) United Nations, World Drug Report, 2020.

[4] H. H. Maurer, Current role of liquid chromatography-mass spectrometry in clinical and forensic toxicology, Analytical and Bioanalytical Chemistry, 388(7) (2007) 1315 – 1325.

[5] S. Chiang, W. Zhang, Z. Ouyang, Paper spray ionization mass spectrometry: recent advances and clinical applications, Expert Review of Proteomics 15(10) (2018) 781 – 789.

[6] S. Chiang, W. Zhang, Z. Ouyang, Paper spray ionization mass spectrometry: recent advances and clinical applications, Expert Review of Proteomics 15(10) (2018) 781 – 789.

[7] C. H. Lin, W. C. Liao, H. K. Chen, T. Y. Kuo, Paper spray-MS for bioanalysis, Bioanalysis 6(2) (2014) 199 – 208.

[8] N. E. Manicke, B. J. Bills, C. Zhang, Analysis of biofluids by paper spray MS: advances and challenges, Bioanalysis 8(6) (2016) 589 – 606.

[9] J. Liu, H. Wang, N. E. Manicke, J. M. Lin, R. G. Cooks, Z. Ouyang, Development, characterization, and application of paper spray ionization, Analytical Chemistry 82(6) (2010) 2463 – 2471.

[10] J. McKenna, R. Jett, K. Shanks, N. E. Manicke, Toxicological Drug Screening using Paper Spray High-Resolution Tandem Mass Spectrometry (HR-MS/MS), Journal of Analytical Toxicology 42(5) (2018) 300 – 310.