Rapid Detection of Drugs-of-abuse in Urine and Oral Fluid by Mass Spectrometry

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ABSTRACT

Development of simple, reliable and cost-effective methods for drug analysis is crucial for the fight against drug abuse. In order to deal with large number of samples, preliminary screening is normally firstly applied, followed by confirmatory analysis. However, the preliminary screening, which involves the use of testing kits, has the problem of producing false positive or false negative results, while the confirmatory analysis is time-consuming and laborious as extensive sample pretreatment and chromatographic separation process are required. To solve these problems, we have developed two techniques, i.e., wooden-tip electrospray ionization mass spectrometry (WT-ESI-MS) and solid phase microextraction coupled with electrospray ionization mass spectrometry (SPME-ESI-MS), for rapid and reliable detection of common drugs-of-abuse in human urine and oral fluid.

Keywords: drug analysis, ambient inoization, wooden-tip electrospray ionization mass spectrometry, solid phase microextraction

1 INTRODUCTION

Drug analysis is an essential task in controlling the abuse of drugs. Due to the prevalence of the drug abuse problem, chemical analysis units are required to handle a large number of body fluid samples for law enforcement and healthcare purposes, with analysis of drugs-of-abuse typically performed with a two-step strategy, i.e., preliminary screening followed by confirmatory analysis [1]. The preliminary screening is commonly performed using the antibody-based on-site screening kits and the immunoassay methods [1-2]. However, generation of false positive and false negative results has frequently been reported for those screening kits. Normally, the samples with postive responses in the screening are further subjected to confirmatory analysis, which involves use of analytical techniques such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) [3-4]. In order to reduce the matrix interference, extraction of drug residues in the samples and further sample clean-up are required, which can be time-consuming and laborious. For these reasons, development of rapid, reliable, and sensitive methods for detection of illicit drugs is an important task in controlling drug abuse, and various techniques have been attempted for this purpose in recent years [5-8].

Wooden-tip electrospray ionization mass spectrometry (WT-ESI-MS) is a technique developed by our group, which is easy-to-setup and allows direct analysis of raw samples [9]. This technique was demonstrated to be applicable in rapid analysis of ketamine (KET) and its metabolite nor-ketamine (NKET) in urine and oral fluid with little sample preparation and no chromatographic separation [6]. The WT-ESI-MS method has been extended for rapid detection of six common drugs-of-abuse, including ketamine, methamphetamine (mAMP), cocaine (COC), methylene dioxymethamphetamine (MDMA), cannabis (THC) and heroin, and their metabolites in urine and oral fluid. Furthermore, we have coupled solid phase microextraction (SPME), a rapid and efficient extraction and enrichment technique [10-12], with ESI-MS (termed as SPME-ESI-MS) for improved detection of drugs-of-abuse in urine and oral fluid.

2 EXPERIMENTAL

2.1 Preparation of drug samples

Different concentrations of drug samples were prepared by spiking known concentrations of drug standard solutions into blank urine and oral fluid. The calibration plots were constructed with at least five different concentrations. The samples for the validation study were prepared at low, middle and high concnetrations of the linear ranges.

2.2 WT-ESI-MS method

Sample preparation for WT-ESI-MS: The urine and oral fluid samples are diluted with two folds of methanol and spiked with the internal standards.

WT-ESI-MS setting: A commercially available wooden toothpick was sharpened and mounted onto the capillary holder of the nano-ESI ion source which was connected to high voltage supply (Figure 1a). An aliquot of 2 μL of each prepared sample solution was applied onto a wooden tip and analyzed with multiple reaction monitoring (MRM) mode. Upon application of a high voltage to the wooden tip, spray ionization was induced and ion signals of the analytes were detected.

2.3 SPME-ESI-MS method

Sample preparation for SPME-ESI-MS: Internal standards were spiked into the raw samples. A pre-

conditioned C18 SPME tip was immersed in 1000 µL of urine or 500 µL of oral fluid sample for extraction of analytes. After 5-10 minutes of extraction, the SPME tip was washed with water for 10 seconds.

SPME-ESI-MS setting: The SPME tip was fixed in front of the mass spectrometer inlet (Figure 1b). With addition of the elution and ionization solvents and application of a high voltage, the extracted analytes could be eluted and sprayed out to be detected. Solvent supply was stopped after 30 seconds in order to obtain desirable signals for quantitative analysis.

Figure 1. Set up of WT-ESI-MS (a) and SPME-ESI-MS (b).

3 RESULTS AND DISCUSSION

3.1 Detection and quantitation of drugs-of-abuse in urine and oral fluid using WT-ESI-MS

The results of methamphetamine are shown in Figure 2. Each "peak" in the chromatogram represented the addition of one aliquot of sample solution onto the wooden tip. The MRM signals of the analyte showed a positive correlation with the analyte concentration, while the signals for the internal standard did not vary significantly. By plotting the intensity ratio of the analyte and internal standard against the spiked concentration of the analyte, a calibration curve with a good linearity for quantitation of methamphetamine in urine was obtained. Similar results were obtained for ketamine, nor-ketamine, methamphetamine, MDMA, cocaine and benzoylecgonine in urine and oral fluid. The accuracy and precision for the analysis of the mentioned drugs in urine and oral fluid were tested by the samples containing with analytes of low, middle and high concentrations in the linear range. The accuracy was 88.4%-122.3% in urine and 81.8%-114.4% in oral fluid and the precision (in term of relative standard deviation) was generally less than 15%.

For the determination of LODs, blank samples were firstly applied onto the wooden tips to obtain the background signals. The LOD was defined as the concentration of the analyte that could produce signal 3 times that of the blank signal (S/N =3). Table 1 showed the LODs of some of the target drugs, which generally fulfilled the recommended cut-off levels of international associations such as EWDTS (2015) and SAMHSA (2010). However, the detection of benzoylecgonine, heroin, 6-monoacetylmorphine, morphine, THC and THC-COOH needed to improve.

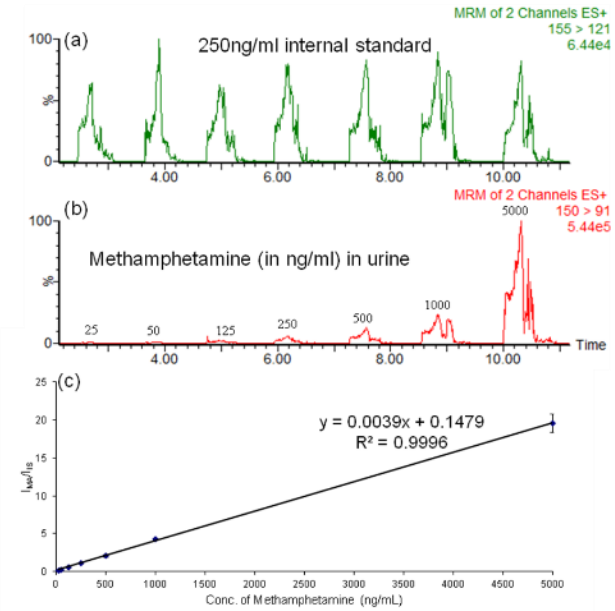

Figure 2. MRM signals of (a) 250 ng ml^{-1} of methamphetamine-D5 internal standard and (b) different concentrations of methamphetamine in spiked urine. (c) A calibration curve obtained for quantitation of methamphetamine in urine.

Table 1. LODs of WT-ESI-MS for detection of drugs, as compared with the international standards (urine/oral fluid, in ng ml^{-1})

Compound	Our result	Cut-off values	
		SAMHSA	EWDTS
KET	20/20	No data	No data
NKET	20/20	No data	No data
mAMP	25/12.5	250/50	200/15
MDMA	50/50	250/50	200/15
COC	12.5/12.5	No data	No data

3.2 Detection and quantitation of drugs-of-abuse in urine and oral fluid using SPME-ESI-MS

Different SPME tips and different extraction time were tested to obtain the optimized results. For the drugs such as ketamine, methamphetamine and MDMA, which can be easily ionized, extraction for five minutes with gentle sharking was enough. Longer extraction time such as ten minutes was required for the extraction of heroin, 6-monoacetylmorphine and morphine. The linear range, linearity, accuracy and precision of SPME-ESI-MS for quantitation of the target drugs were tested as well, in a way similar to the WT-ESI-MS study. Our results showed that all these parameters obatined were acceptable for the analysis. The LODs of the drugs were also determined and some of

the data are shown in Table 2. The LODs of SPME-ESI-MS for detection of the target drugs were greatly improved, compared with those of the WT-ESI-MS. It was noteworthy that 6-monoacetylmorphine and morphine, which were poorly detected using WT-ESI-MS, could be detected using SPME-ESI-MS with acceptable LODs . The improvements was believed to be due to the enrichment of the analytes onto the SPME tip and the reduction of the matrix interference caused by the extraction and washing process. However, the detection of those poorly ionized compounds, especially THC, was still not comparable with that of LC-MS. Developing new SPME tips for more selective enrichment of those analytes may allow improved results.

Table 2. LODs of SPME-ESI-MS for detection of drugs, as compared with the international standards (urine/oral fluid, in ng ml^{-1})

Compound	Our result	Cut-off values	
		SAMHSA	EWDTS
KET	10/5	No data	No data
NKET	5/2	No data	No data
mAMP	2/2	250/50	200/15
MDMA	2/2	250/50	200/15

4 CONCLUSION

Two different techniques, i.e. WT-ESI-MS and SPME-ESI-MS, have been established and demonstrated to be applicable for rapid detection and quantitation of drugs-of-abuse in urine and oral fluid. The linearity, accuracy and precision were tested and the results were desirable. WT-ESI-MS could be applied for direct analysis of ketamine, nor-ketamine, methamphetamine and MDMA in urine and oral fluid. On the other hand, SPME-ESI-MS allowed sensitive and rapid detection of the target drugs in urine and oral fluid.

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Journal paper related to a part of this study has been published by the authors (So et al., Analyst, 138, 2239-2243, 2013). This is the continued work of the previous study.

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