Beat Drugs Fund

Full Report

Project title: Enhanced Detection and Quantitation of Drugs-of-abuse in Urine and Oral Fluid by Solid Phase Microextraction (SPME) Coupled with Mass Spectrometry

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1. Introduction

Drug abuse is a serious problem in Hong Kong nowadays. Drug analysis is an essential task in controlling of drug abuse. Due to the prevalence of the drug abuse problem, chemical analysis units are required to analyze a large number of body fluid samples for law enforcement and healthcare purposes, and typically a two-step strategy, i.e., preliminary screening followed by confirmatory analysis, is used for drug analysis.[1-3] Preliminary screening for the presence of illicit drug residues in body fluids is commonly performed by on-site antibody-based screening devices and immunoassay methods.[1, 3-8] However, these methods possess a variety of problems, including cross-reactivity and generation of false positive and false negative results.[3-4, 6-9] Therefore, it is a common practice that positive samples screened out in preliminary screening are further subjected to confirmatory analysis using analytical techniques such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS).[1, 3-4, 10-15] However, these techniques typically require extensive sample preparation procedures, e.g., liquid-liquid extraction and solid-phase extraction, for reduction of matrix interference and sample enrichment that could be time-consuming and laborious. For the above reasons, development of rapid, reliable and sensitive methods for analysis of illicit drugs has been an important task in controlling drug abuse. In our previous Beat Drugs Fund project (BDF120020), wooden-tip electrospray

ionization mass spectrometry (WT-ESI-MS) was developed for rapid and simple analysis of drugs-of-abuse in urine and oral fluid. However, the sensitivities for analysis of some of the targeted analytes, such as benzoylecgonine, morphine and THC, were still not good enough by using WT-ESI-MS.

Solid-phase microextraction (SPME) is a rapid and efficient extraction and enrichment technique which was invented by Arthur and Pawliszyn in 1990.[16] This technique makes use of a micro-tip, usually silica-based tip, coated with various materials on the tip surface for selective extraction and enrichment of analytes in raw samples. By selecting an appropriate coating material, analytes can be selectively retained and enriched on the tip and the interfering matrices can be washed out.[17,18] In this project, SPME was used to replace the wooden tips to allow rapid and effective extraction and enrichment of drugs-of-abuse in urine and oral fluids and then direct coupling with mass spectrometry (SPEM-ESI-MS) for rapid analysis. The SPME-ESI-MS method was developed for rapid and sensitive analysis of six common abused drugs, i.e., ketamine, methamphetamine, cocaine, ecstasy (MDMA), cannabis (THC) and heroin, and their metabolites in urine and oral fluids. The coupling of SPME with commercially available portable GC-MS for on-site analysis of drugs-of-abuse was also investigated in this project.

2. Methodology

2.1 Instrumental setup

The instrumental setup for SPME-ESI-MS is illustrated in Figure 2-1. An Agilent 6460 triple quadrupole mass spectrometer equipped with ultra-performance liquid chromatography (UPLC) system (Santa Clara, CA, USA) was used in this study. An external high voltage supply (Hengbo Electric Co. Ltd., Zhejiang, China) with 3.5 kV for both the positive and negative ionization mode was used for the ESI. The capillary voltage on the instrument side was set to 100 V, the source temperature was 150 ^{o}C and the source gas flow was 6 L/min. The mass spectrometer was operated under MRM mode. The MRM channels for the detection and quantitation of the targeted analytes are listed in Table 2-1. The Dwell time of each channel was 100 ms. A home-built platform was placed in front of the mass spectrometer inlet for affixing the SPME tip for SPME-ESI-MS. The platform was consisted of a stand and clip for adjusting the height, a glass slide fixed by the clip for supporting the SPME tip and a cushion fixed at the edge of the glass slide for fixing the SPME tip in position. A sprayer which is an original ESI nebulizer was removed from the Agilent's mass spectrometer and loaded with spray solvents for eluting and ionizing the analytes absorbed or adsorbed onto the SPME tip. The sprayer was pointed toward the SPME tip. The solvent was supplied by a programmable syringe pump (New Era Pump System Inc., Farmingdale, NY, USA) with the flow rate of 30 μL/min and the nitrogen gas flow of 3 psi. The sprayer was

grounded to protect the operator.

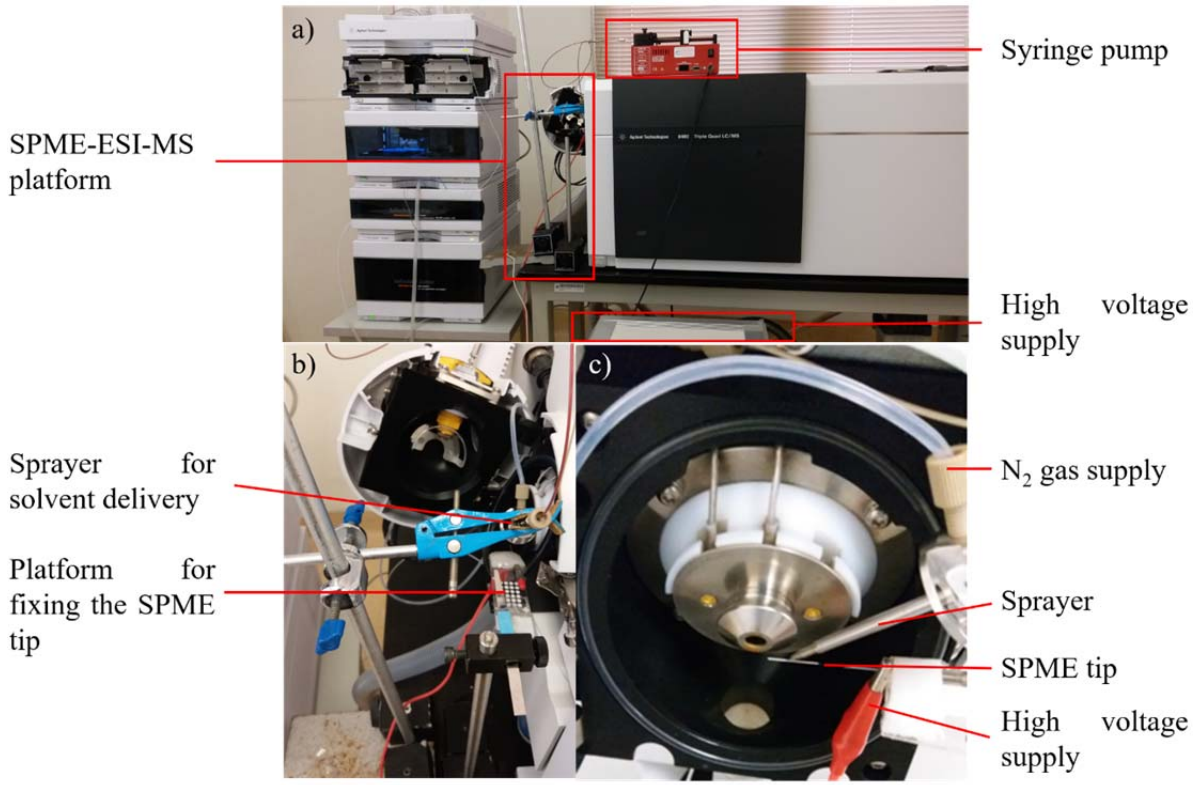

Figure 2-1. (a) The setup of SPME-ESI-MS on an Agilent 6460 triple quadrupole mass spectrometer. (b) Close-up of the platform placed in front of the mass spectrometer for SPME-ESI-MS analysis and (c) Close-up of a SPME tip mounted onto the platform for SPME-ESI-MS analysis.

Table 2-1. MRM condition and fragmentor setting of various drugs, metabolites and deuterium labeled internal standards.

Analyte	MRM Channel	Collision cell energy (V)	Fragmentor (V)
Ketamine (KET)	238 → 125*	22	80
	238 → 220	10	80
Nor-ketamine (Nor-K)	224 → 125*	20	80
	224 → 207	10	80
Ketamine-D_4 (D-KET)	242 → 129	22	80
Nor-ketamine-D_4 (D-Nor-K)	228 → 129	20	80
Methamphetamine (MA)	150 → 91*	17	80
	150 →119	9	80
Methamphetamine-D_5 (D-MA)	155 → 121	9	80
MDMA	194 → 163*	8	80
	194 → 105	22	80
MDMA-D_5 (D-MDMA)	199 → 165	8	80
Cocaine (COC)	304 → 182*	15	120
	304 → 82	28	120
Cocaine-D_3 (D-COC)	307 → 185	15	120
Benzoylecgonine (BEN)	290 → 168*	15	120
	290 → 105	28	120
Benzoylecogonine-D_3 (D-BEN)	293 → 171	15	120
THC	315 → 193*	18	120
	315 → 123	32	120
THC-D_3	318 → 196	20	120
THC-COOH	343 → 299*	15	200
	343 → 245	25	200
THC-COOH-D_9	352 → 308	15	180

(To be continued)

Heroin (HER)	370 → 165*	55	200
	370 → 211	30	200
Heroin-D_9 (D-HER)	379 → 165	55	180
6-monoacetylmorphine (6-MAM)	328 → 165*	40	140
	328 → 211	25	140
6-monoacetylmorphine-D_3 (6-MAM)	331 → 165	40	140
Morphine (MOR)	286 → 165*	48	180
	286 → 153	48	180
Morphine-D_3 (D-MOR)	289 → 165	45	180

*The channels used for quantitation of the analyte.

2.2 SPME-ESI-MS workflow

The C18 SPME tips were wetted with 1 mL 1:9 (v/v) H_2O/MeOH for 10 min and conditioned with 9:1 (v/v) H_2O/MeOH for another 10 min before extraction. Related internal standards were spiked into the urine and oral fluid samples. The SPME tips were immersed into the urine and oral fluid samples for 5 min for the extraction with vortex on a Bench Mixer at ~200 rpm (Benchmark Scientific Inc., Edison, NY, USA). The extraction time for heroin, 6-acetylmorphine, morphine, THC and THC-COOH, was 10 min. The SPME tips were rinsed with water for 10 s and ready for SPME-ESI-MS analysis. After the extraction, a SPME tip was affixed at 90^o in front of the mass spectrometer (0.6 - 0.8 cm horizontally and 0.4 – 0.8 cm vertically away from the mass spectrometer) through the SPME-ESI-MS platform. The high voltage supply was connected to the SPME tip. The spray solvent, which acted as both

the elution and ionization solvent, was delivered onto the SPME tip through a sprayer (~0.5 cm away from and ~65^{o} pointed to the middle part of the SPME tip). A ratio of 10:90:0.1 (v/v/v) H_2O/EtOH/FA was used as the spray solvent except 10:90:0.1 (v/v/v) H_2O/MeOH/FA was used for the analysis of heroin, 6-acetylmorphine and morphine. The syringe pump was stopped after 10 μL of solvent was applied onto the SPEM tip. The MRM signal could generally last for 10 – 20 s. For each SPME tip, the solvent was applied onto the SPME tip for three times and three data were recorded. The residues on the SPME tips were removed by washing the tip with 90:10:0.1 (v/v/v) MeOH/H_2O/FA at 40 ^{o}C for 15 min twice. The C18 SPME tips could generally be reused for 10 times. The MRM spectra were analyzed using Agilent Qualitative Analysis software.

2.3 Method validation of SPME-ESI-MS

Calibration curves

The calibration curves for quantitation were constructed by averaging three sets of experimental data, while each set of data was obtained by analyzing at least five different concentrations of analytes. The resultant MRM chromatograms were processed using Agilent Qualitative Analysis software. The signals were manually integrated, and the peak areas were used for constructing the calibration curves. The targeted drugs and its metabolites in the same groups were analyzed at the same experiments, i.e. four experiments were performed

for constructing the calibration plots of the targeted drugs.

Accuracy and precision

The accuracy and precision of SPME-ESI-MS method was determined by using urine and oral fluid samples spiked with the analytes at low, medium, and high concentrations respectively. Samples at each concentration were analyzed at least six times and the data obtained were averaged for comparison. The accuracy was calculated by:

$$\frac{\text{concentration of analyte determined}}{\text{actual concentration of the analyte in the sample}} \times 100\% \qquad (2\text{-}1)$$

and the precision, i.e., relative standard deviation (R.S.D.), was calculated by:

$$\frac{\text{standard deviation of the concentration determined}}{\text{mean of the concentration determined}} \times 100\% \qquad (2\text{-}2)$$

Limit-of detection (LOD) and limit-of-quantitation (LOQ)

Blank samples were prepared by spiking only the internal standards into blank urine or oral fluid. The LODs and LOQs were determined by comparing the peak area ratio of the analytes and internal standards between the spiked samples and the blank samples. The determination of LOD was followed the definition of IUPAC,[19] which is given by the equation:

$$x_L = \bar{x}_{bi} + k\, s_{bi} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ (2\text{-}3)$$

where x_L is the smallest measure (signal) that can be detected with reasonable certainty, $\bar{x}_{bi}$ is the mean of the blank measures, s_{bi} is the standard deviation of the blank measures

and k is a numerical factor. The LOD and LOQ of an analyte were defined as the concentrations of the spiked samples that can give signal (relative peak area) larger than x_L with the k equal to 3 and 10 respectively. At least nine measurements of the blank and the spiked samples were obtained for the determination of LODs and LOQs.

2.4 SPME potable GC-MS (SPME-p-GC-MS) analysis

A Mars-400 plus portable GC-MS (Focused Photonics Inc., Hangzhou, China) equipped with DB-5MS GC column (5 m x 0.1 mm x 0.4 μm) and ion trap, was used in this study. The SPME tip (with 65 μm PDMS/DVB coating) was immersed into 1.5 mL sample for solid phase microextraction for 15 min under magnetic stirring and heating at 50^{o}C. After extraction, the SPME tip was then inserted into the portable GC-MS for GC-MS analysis as shown in Figure 2-2. The GC-MS setting was: Injector temperature 260 ^{o}C, transfer line temperature 200 ^{o}C, ion trap temperature 150 ^{o}C, helium gas flow rate 0.2 mL/min, and the temperature program: 120 ^{o}C at the first 3 min, increased to 240 ^{o}C at the rate of 60 ^{o}C/min and reached 300 ^{o}C with the rate of 20 ^{o}C/min, and held for 5 minutes. The total run time for the GC-MS analysis was 13 min. The MS was operated at scan mode (scan range from *m/z* 45 to *m/z* 500). The whole SPME-p-GC-MS could be finished within 30 min. The SPME tip could be re-used after the analysis.

Figure 2-2. Overview of the portable GC-MS instrument used in this study.

2.5 Chemical derivatization of drugs-of-abuse

The procedures of derivatization of THC and THC-COOH were modified from Musshoff et al. (2002).[20] 0.5g of sodium bicarbonate (Na_2CO_3) was firstly added to 1 mL urine sample. The analytes in the sample were extracted by PDMS and PDMS/DVB SPME tips for 25 min using HS extraction. The SPME tips were then exposed in vials with 25 µL N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) at 90^{o}C for 8 min. The SPME tips were then inserted into portable GC-MS for GC-MS analysis.

Similar procedures were used for the derivatization of remaining drugs-of-abuse with modifications. Instead of MSTFA, 50 µL pentafluoropropionic anhydride (PFPA) and 25 µL 2,2,3,3,3-pentafluoro-1-propanol (PFPOH) were used for the derivatization, and the reaction were performed at 70 ^{o}C for 20 min.

2.6 Method validation of SPME-p-GC-MS

Calibration curves

The calibration curves for quantitation were constructed by averaging three sets of experimental data, while each set of data was obtained by analyzing at least five different concentrations of analytes with fixed concentration of D-THC as the internal standard. The extracted chromatogram of most abundant fragment ion (*m/z* = 299 for THC) was used to construct the calibration curves. The signals were manually integrated, and the peak areas were used.

Accuracy and precision

The accuracy and precision of SPME-ESI-MS method was determined by using urine samples spiked with the analytes at low, medium, and high concentrations respectively. Samples at each concentration were analyzed at least three times and the data obtained were averaged for comparison. The accuracy and precision were calculated same as equation 2-1 and 2-2.

Limit-of detection (LOD) and limit-of-quantitation (LOQ)

The LOD and LOQ were defined as the quantity of analyte that could achieve a signal-to-noise (S/N) ration of the most abundant fragment ion with the factors of three and

ten, respectively.

3. Results and discussions

3.1 Optimization of the SPME-ESI-MS protocol

Optimization of analyte extractions

Optimization of extraction conditions of targeted drugs is a crucial step for successful SPME-ESI-MS analysis, as the sensitivity of the detection is highly dependent on the amount of analytes enriched on the SPME tip. Four parameters including selection of the SPME tip coatings, extraction time, extraction pH and addition of salt were optimized for the extraction of each targeted analytes.[21]

Selection of SPME tip coatings is an important step for the optimization of SPME protocol There are four SPME coatings including PDMS, PDMS/DVB, polyacrylate (PA) and C18 available for LC analysis. PDMS is suitable for the extraction of non-polar volatiles, PA is normally used for the extraction of polar compounds and DVB/PDMS and C18 are more universal, which can be used for the extraction of semi-polar analytes. All of the above SPME tips were tested to extract each targeted analyte in this study. Both C18 and DVB/PDMS SPME tips could extract KET, Nor-K, MDMA and MA in urine effectively while the performance of C18 was slightly better than DVB/PDMS SPME tip. The extraction using

PDMS and PA SPME tip gave very low extraction efficiency. The results obtained from other targeted analytes were similar, both C18 and PDMS/DVB SPME tips could be used for the drug extraction. However, considering the higher extraction efficiency of C18 SPME tip for the extraction of THC-COOH, BEN and 6-MAM than that of PDMS/DVB SPME tip, C18 SPME tip was finally selected in this study.

The extraction time from 1 min to 40 min were tested to optimize the experimental protocol The result showed that the extraction rates of KET, Nor-K, MDMA and MA were fastest at 5 – 20 min and reached a plateau after 40 min and similar results were obtained for the extraction of other analytes. Logically, the longer the extraction time, the more the amount of analytes are extracted onto the SPME tip. However, the purpose of this study is to develop a method for rapid analysis of drugs-of-abuse, extraction for more than 40 min is too long for a rapid detection method. Therefore, to balance between the extraction time and the analytical performance, the extraction time for the targeted analytes was set as the shortest time that sufficient amount of analytes were extracted to fulfill the cut-off level of the international standards. For KET, Nor-K, MDMA, MA, COC and BEN, 5 min extraction was enough for the resultant LODs reaching the cut-off of international standards. For HER, 6-MAM, MOR, THC and THC-COOH, 10 min extraction was required to fulfill the analytical requirements.

Urine samples at pH 5, 7 and 9 were tested to optimize the pH for the extraction. No further pH values were tested as extreme pH may damage the SPME tips. Most of the targeted analytes such as KET, Nor-K, MDMA and MA favored the extraction when pH ≥ 7, except THC-COOH which was better extracted at pH 5. Sample extraction was normally set at pH 7 in this study as most of the drugs had the optimized extraction at pH 7. Since the pH value of human urine is between 5.5 – 7 which could slightly reduce the extraction efficiency of SPME,[22] measuring the pH of urine samples and adjusting the pH is necessary. In contrast, the pH value of oral fluid is between 6.5 – 7.2, and therefore pH adjustment is generally not essential.[23]

Finally, the salt concentration in the sample solution was optimized. In most of the applications, high salt content can increase the extraction efficiency of SPME through salting-out effect. However, the addition of salt may not improve the performance of SPME in some cases, for example there is no effect for highly polar compounds or compounds with high water solubility. For some cases, addition of NaCl even reduces the performance of SPME as more impurities in the sample solution will also be extracted.[21] No significant improvement was observed for the extraction of all the targeted analytes after the addition of NaCl. The results obtained here are consistent with the study done by Chou and Lee.[24] The possible explanation for this is the addition of salt also increased the extraction of matrix

components which did not benefit the extraction of targeted analytes.

In summary, the optimized extraction was obtained by using C18 SPME tip with 5 – 10 min extraction under vortex. The samples solution was adjusted to pH 7 and addition of salt was not necessary. For urine samples, 1mL sample was used as the extraction was done in a 1.5 mL eppendorf. On the other hand, 500 μL sample was used for oral fluid sample as the collection volume of oral fluid was limited, and some of the oral fluid devices could only collect about 500 μL of oral fluid.[25]

Optimization of the SPME-ESI-MS setup

There are several parameters that could affect the performance of SPME-ESI-MS including the spray solvent delivery methods, the distance between the SPME tip and the MS ion inlet and the spray solvent compositions. The above parameters were tested.

Three different spray solvent delivery methods were tried for SPME-ESI-MS including the addition of solvent using pipette, syringe pump and sprayer. In the pipette method, the solvent was added onto the SPME tip similar to that of WT-ESI-MS. On the other hand, the syringe pump method is described by Ahmad and co-workers[25], which the solvent is continuously supplied by syringe pump. However, the signals obtained from these methods were poor. The

signals were discontinuous and the signal durations were very short. It was observed that a big solvent droplet was accumulated onto the SPME tip rather than sprayed out in syringe pump method. Therefore, a sprayer with solvent supply from the syringe pump was used for the solvent delivery instead. Finer solvent droplet created by the sprayer was landed onto the SPME-tip and solvent accumulation was prevented, resulting of the production of continuous signal. It is not reasonable and necessary to spend longer than 10 min to record one signal. In the final SPME-ESI-MS setup, the solvent supply to the sprayer was stopped after the signal reached its maximum, which was around 20 s (equivalent to 10 μL of solvent at the flow rate of 30 μL/min).

The distance between SPME tip and the MS ion inlet were also optimized. The distance was started from 3 x 3 cm (horizontal distance x vertical distance) away from the mass spectrometer. An external high voltage supply was connected to the SPME tip for the ESI. The SPME tip was moved 0.5 x 0.5 cm forward to the ion inlet each time and finely adjusted when strong signal was observed. MRM signals were observed when the distance was set to 1.5 x 1.5 cm and strongest signals were observed for the distance with 0.6 - 0.8 x 0.4 - 0.8 cm.

The spray solvent plays an important role for the elution and ionization of the targeted

analytes from the SPME tip. Different organic solvent and water solvent systems were tested for SPME-ESI-MS. Generally, the higher the ratio of the organic solvent, the higher the signal intensity was observed. 10% of water was still kept in the spray solvent as it is necessary to wet the surface of SPME tip in order to elute and ionize the analytes and to prevent the spray solvent evaporation before it reached the SPME tip. Various organic solvents were also tested. Generally, strongest signals were produced by using EtOH as the spray solvent, followed by using MeOH. However, the signals obtained by using ACN were significantly lower than that of EtOH and MeOH. 90% EtOH was the most effective spray solvent for SPME-ESI-MS for most of the drugs, except for HER, MOR and 6-MAM, which 90% MeOH could give better signals. Therefore, 90% EtOH was selected as the spray solvent at normal situation except 90% MeOH was used for detection of heroin and its metabolites.

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

Detection of drugs-of-abuse

Typical MRM results for the detection of ketamine in urine are shown in Figure 3-1a. The spray solvent was applied onto the SPME tip for 20 s each time. Typically, the spray solvent was applied onto the same SPME tip 3 times to ensure the stability of the signals. Signals were considered as positive only if the S/N of the quantifier ion channels is $\geq$ 3 when

compared with that of the blank, as shown in Figure 3-1b. Also, the presence of the analytes was further confirmed by monitoring an additional qualifier ion channel for each targeted analyte. The ion ratio between the quantifier ion and qualifier ion should be within certain value as suggested by EWDTS if a particular drug is present in the samples.

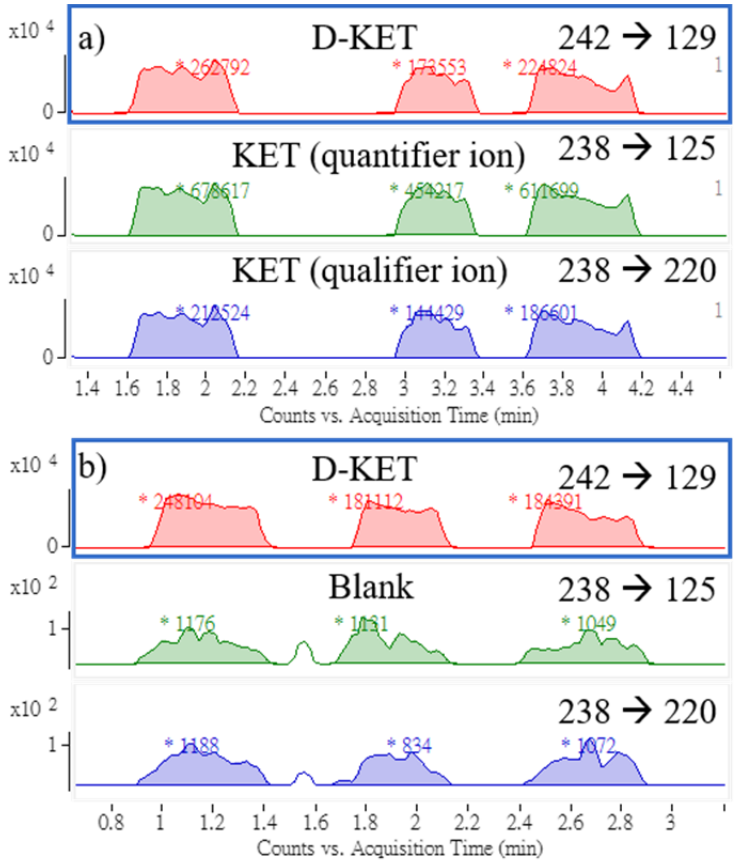

Figure 3-1. Typical MRM results of the detection of (a) 500 ng/mL ketamine in urine and (b) blank urine using SPME-ESI-MS.

Reproducibility of the SPME-ESI-MS method

Samples with the same concentration were repeatedly measured with three individual SPME-ESI-MS at the same day and the whole experiment was repeated on another day and

the results are shown in Figure 3-2. The precisions of the 3 measurements, in term of relative peak areas, using the same SPME tip (no.1 - no.4) were 2.7%, 1.0%, 6.2% and 2.2% respectively and the overall precision (n = 12) was 5.1%. The results demonstrated that the extraction and detection of targeted analytes using the established protocol could be reproducible even the samples were extracted using different C18 SPME tips.

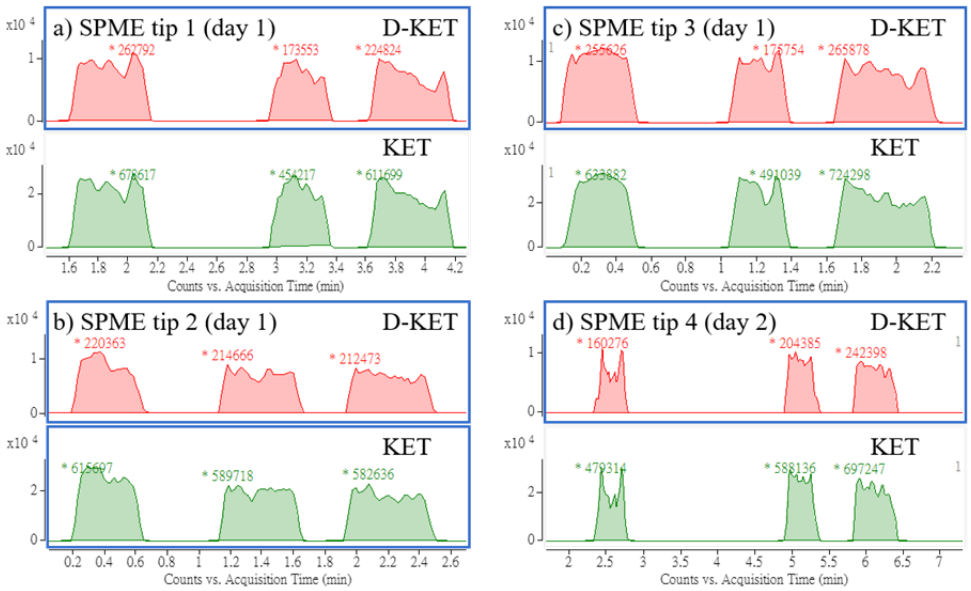

Figure 3-2. MRM results for the detection of 500 ng/mL ketamine in urine using SPME-ESI-MS. (a-c) Repeated experiments using three individual SPME-ESI-MS within the same day and (d) repeated experiment on another day.

Reusability of the C18 SPME tip

The extraction of targeted analytes was achieved using C18 SPME tip. However, the cost of

each C18 SPME tip was around HK$ 180 which is relatively expensive compared with other extraction techniques. Therefore, it would be more cost effective if the C18 SPME tip could be re-used after one analysis. By comparing the results obtained for analyzing blank urine, 1000 ng/mL ketamine in urine and the washed C18 SPME tip after the analysis, the signals were greatly reduced after washing. It indicated that most of the ketamine residue left on the SPME tip after the SPME-ESI-MS analysis could be removed by washing the tips with organic solvent for 30 min. The residue level after washing was only slightly higher than that of the blank. The ion ratio for the detection of ketamine of the SPME tip after washing was also higher than the acceptable value, which could be considered as no ketamine presence on that C18 SPME tip. Therefore, the results showed that C18 SPME tip can be used after proper washing. In this study, each SPME tip can be generally re-used for at least 10 analyses.

Quantitation of targeted analytes

The calibration curves for the quantitation of targeted analytes in urine and oral fluid were constructed by measuring the signals of spiked samples with at least five different concentrations. The calibration curves constructed for each analyte in urine and oral fluid are shown in Figure 3-3 and Figure 3-4 respectively. All the targeted analytes including THC and THC-COOH which were failed to be analyzed using WT-ESI-MS showed linear correlation between the concentrations and signals obtained in SPME-ESI-MS. Linear calibration plots

for quantitation of all targeted analytes were constructed.

The linear range, linearity (in term of R^2) and average R.S.D. of the calibration points of all the targeted analytes in urine and oral fluid are recorded in Table 3-1. Generally, the linear range could nearly cover the range of 10 – 1000 ng/mL except that of THC and THC-COOH. It is due to the signals produced by THC and THC-COOH were unstable at low concentrations, thus the calibration points at low concentrations became nonlinear. Calibration points at higher concentrations were not tested as it is difficult to remove the drug residues on the C18 SPME tips at high concentrations. The R^2 of the curves were greater than 0.99 which indicated good linearity. The reproducibility of relative intensities of each analyte was generally better than 15% except for THC which was less reproducible. The signals of THC were relatively poorer than most of the analytes and its signals thus were not stable at low concentration. The results showed that the present method is suitable for quantitation of drugs-of-abuse in urine and oral fluid and the performance is better than that of WT-ESI-MS.

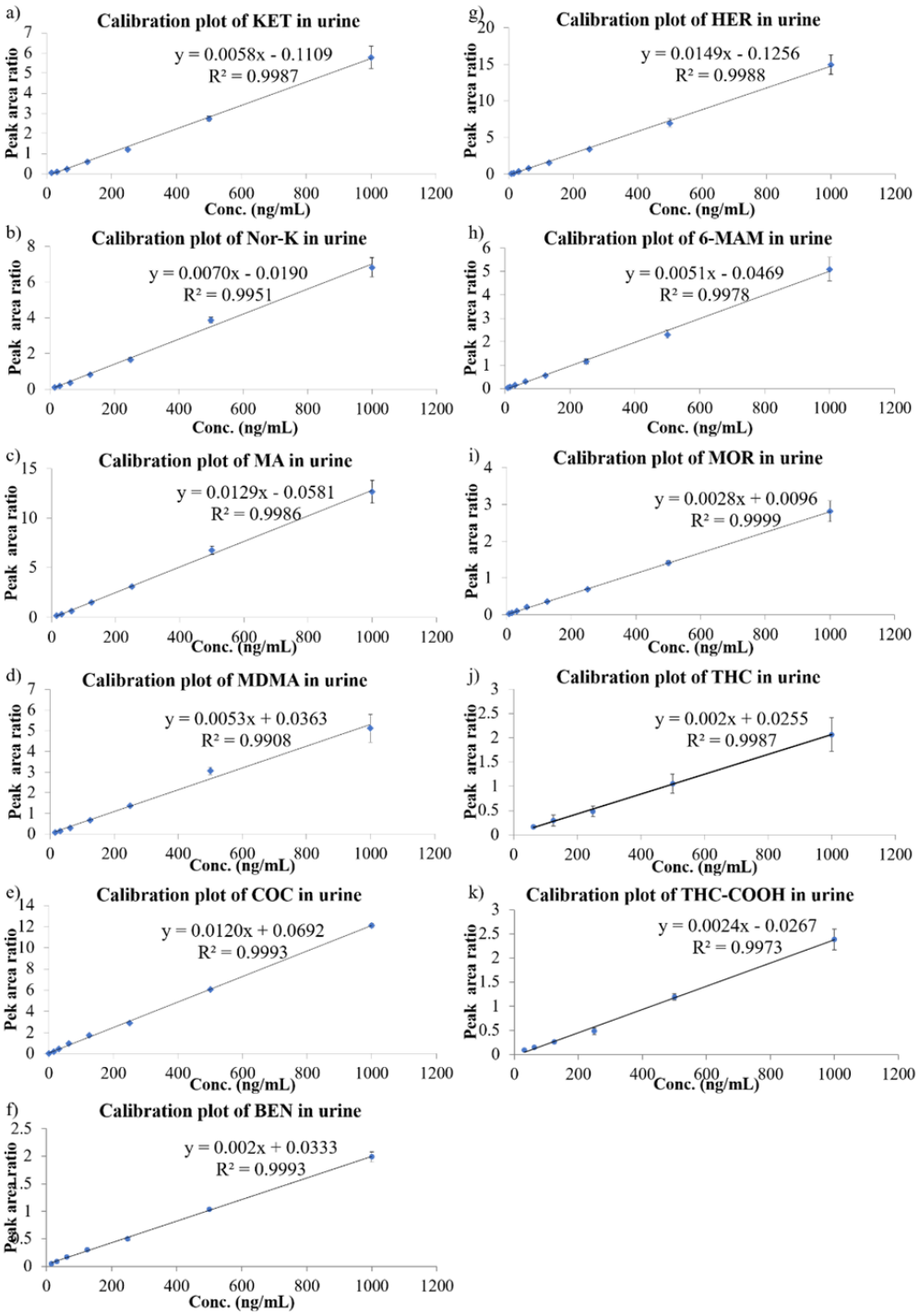

Figure 3-3. Calibration plots for the quantitation of (a) ketamine, (b) nor-ketamine, (c) methamphetamine, (d) MDMA, (e) cocaine, (f) benzoylecgonine, (g) heroin, (f) 6-monoacetylmorphine, (i) morphine, (j) THC and (k) THC-COOH in urine.

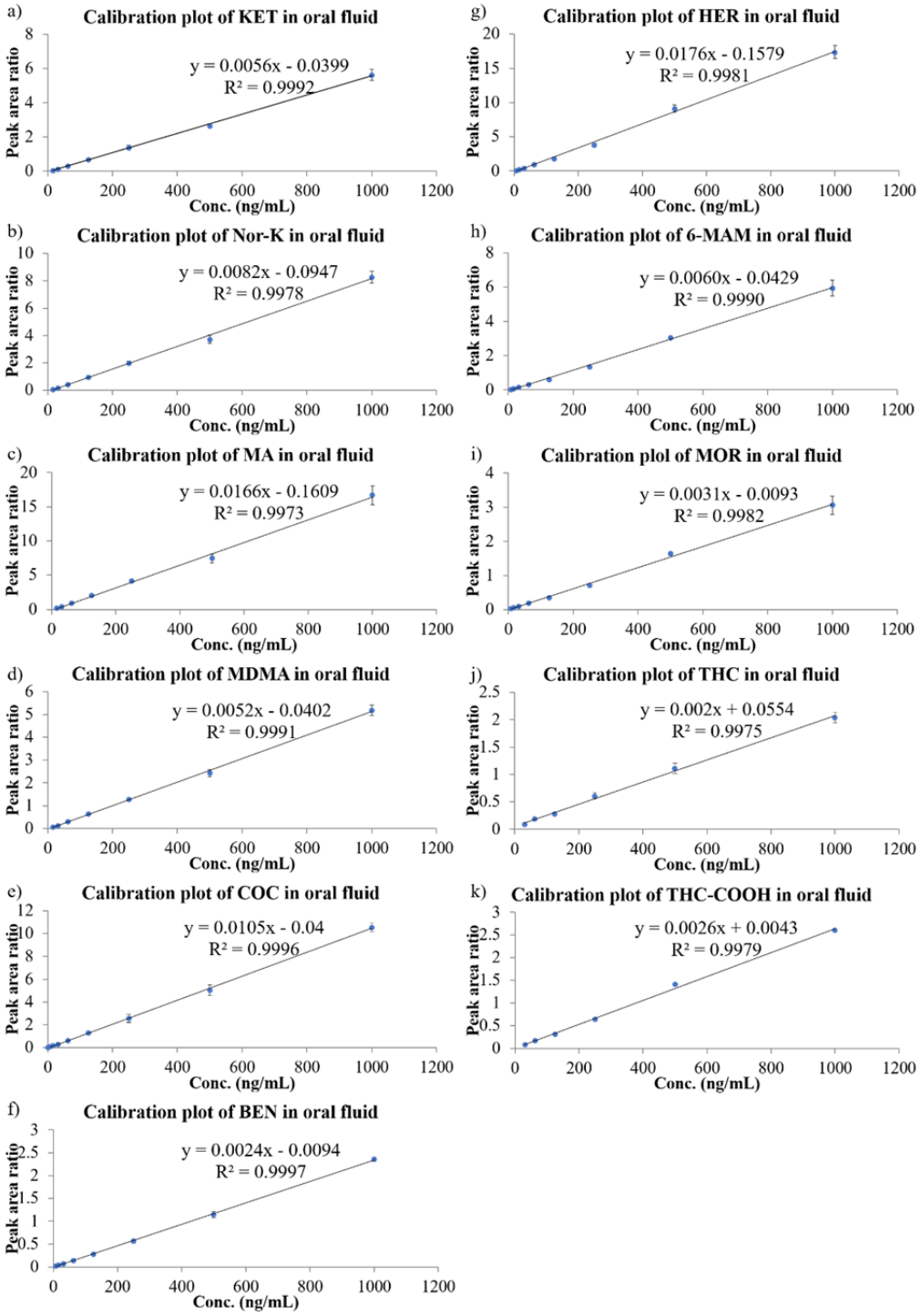

Figure 3-4. Calibration plots for the quantitation of (a) ketamine, (b) nor-ketamine, (c) methamphetamine, (d) MDMA, (e) cocaine, (f) benzoylecgonine, (g) heroin, (f) 6-monoacetylmorphine, (i) morphine, (j) THC and (k) THC-COOH in oral fluid.

Table 3-1. Linearity of targeted drugs and metabolites in urine and oral fluid**.**

Compound		Linear range (ng/mL)	R^2	Average R.S.D. of calibration points (%)
Ketamine	Urine	15.6-1000	0.9987	6.0
	Oral fluid	15.6-1000	0.9992	14.3
Nor-ketamine	Urine	15.6-1000	0.9951	7.1
	Oral fluid	15.6-1000	0.9978	15.5
Methamphetamine	Urine	15.6-1000	0.9986	7.9
	Oral fluid	15.6-1000	0.9973	13.3
MDMA	Urine	15.6-1000	0.9908	7.5
	Oral fluid	15.6-1000	0.9991	12.7
Cocaine	Urine	0-1000	0.9993	4.2
	Oral fluid	0-1000	0.9996	11.7
Benzoylecgonine	Urine	15.6-1000	0.9993	5.6
	Oral fluid	7.8-1000	0.9997	11.9
Heroin	Urine	7.8-1000	0.9988	6.8
	Oral fluid	7.8-1000	0.9981	8.9
6-monoacetylmorphine	Urine	7.8-1000	0.9978	8.6
	Oral fluid	7.8-1000	0.9990	8.4
Morphine	Urine	7.8-1000	0.9999	9.1
	Oral fluid	7.8-1000	0.9982	9.8
THC	Urine	62.5-1000	0.9987	22.4
	Oral fluid	31.3-1000	0.9975	10.1
THC-COOH	Urine	31.3-1000	0.9973	9.4
	Oral fluid	31.3-1000	0.9979	12.9

Accuracy and precision of quantitative analysis

Spiked quality control samples at low, middle and high concentrations of the targeted analytes in urine and oral fluid were tested. The accuracy and precision of the quantitative analysis results are summarized in Table 3-2. The accuracy for determining the targeted drugs was satisfactory (within 80 – 120%), except the determination of quality control samples at the lowest concentration, which was 73.4 – 162.9%. For the quantitation of the targeted analytes at high and middle concentrations (i.e. 800 ng/mL and 500 ng/mL), the precision was generally within 15%, except for THC in urine (19.5 – 22.7%) which was slightly higher than other values. The performance of the quantitation of drugs at low concentrations (e.g. 50 ng/mL and 20 ng/mL) varied and was generally within 25%. Overall, the results of quantitation of all the targeted analytes at different concentrations were desirable and improved when compared with that of WT-ESI-MS. It is possible to determine the concentration of heroin and related compounds and THC and THC-COOH at low concentrations (e.g. 50 ng/mL and 20 ng/mL) using SPME-ESI-MS. However, the performance for the determination of drugs at low concentration could be improved. One possible solution is to construct calibration curves which only cover the low concentration range, for the determination of drugs at low concentrations.

Table 3-2. Accuracy and precision for analysis of different drugs in urine and oral fluid.

Compound	Spiked quantity (ng/mL)		Determined quantity± S.D. (ng/mL) (n=6)		Accuracy (%)		R.S.D (%)	
	Urine	O.F.	Urine	O.F.	Urine	O.F.	Urine	O.F.
KET	769.2	769.2	605.8±39.1	901.7±62.2	78.8	117.2	6.5	6.9
	487.8	476.2	450.0±33.5	476.2±46.0	92.2	119.9	7.4	8.1
	48.8	47.6	49.2±12.6	58.0±5.4	100.7	121.9	25.5	9.3
	19.8	19.6	15.9±2.1	25.5±3.0	80.4	130.0	13.0	11.7
Nor-K	769.2	769.2	647.2±64.2	670.9±85.6	84.1	87.2	9.9	12.8
	487.8	476.2	475.6±62.5	436.4±45.6	97.5	91.6	13.1	10.4
	48.8	47.6	57.2±10.4	38.5±6.6	117.1	80.9	18.2	17.2
	19.8	19.6	19.0±3.9	14.4±3.5	95.8	73.5	20.3	24.1
MA	769.2	769.2	700.1±62.3	805.6±50.2	87.5	104.7	8.9	6.2
	487.8	476.2	544.7±70.5	549.9±58.9	111.7	115.5	12.9	10.7
	48.8	47.6	46.1±5.4	59.2±5.7	94.5	124.4	11.7	9.6
	19.8	19.6	14.5±3.7	31.9±2.7	73.4	162.9	25.4	8.4
MDMA	769.2	769.2	613.2±34.5	853.0±110.3	79.7	110.9	5.6	12.9
	487.8	476.2	417.2±34.9	460.1±52.3	85.5	96.6	8.4	11.4
	48.8	47.6	49.0±8.2	57.5±9.3	100.4	120.8	16.8	16.2
	19.8	19.6	17.5±2.7	28.5±4.5	88.2	145.2	15.4	15.8
COC	769.2	740.7	661.5±45.3	858.2±45.9	86.0	115.9	6.8	5.4
	487.8	476.2	421.2±14.4	554.8±18.6	86.3	116.5	3.4	3.4
	48.8	47.6	51.8±1.7	54.0±1.1	106.1	113.4	3.3	2.0
	19.8	19.6	23.0±1.0	23.2±0.3	116.3	118.6	4.4	1.3
BEN	769.2	740.7	664.9±48.7	823.0±74.9	86.4	111.1	7.3	9.1
	487.8	476.2	414.7±20.4	533.4±18.0	85.0	112.0	4.9	3.4
	48.8	47.6	43.4±3.6	56.5±2.2	89.0	118.7	8.3	3.8
	19.8	19.6	20.9±6.5	24.5±1.3	105.5	125.0	31.2	5.2
HER	769.2	740.7	817.0±32.1	725.4±68.9	106.2	97.9	3.9	9.5
	487.8	476.2	502.2±63.0	445.5±48.2	103.0	93.6	12.6	10.8
	48.8	48.7	47.1±4.3	48.5±2.7	96.4	99.6	9.0	5.5
	19.8	19.6	26.0±1.9	24.5±1.6	131.2	125.2	7.3	6.6

(To be continued)

6-MAM	769.2	740.7	769.5±63.3	658.2±55.0	100.0	88.9	8.2	8.4
	487.8	476.2	489.4±57.0	441.7±50.4	100.3	92.7	11.6	11.4
	48.8	48.7	48.9±4.1	46.8±3.0	100.3	96.2	8.4	6.5
	19.8	19.6	26.1±2.0	24.3±2.3	132.1	124.2	7.8	9.5
MOR	769.2	740.7	744.8±39.9	634.3±71.4	96.8	85.6	5.4	11.3
	487.8	476.2	476.6±78.1	426.2±50.1	97.7	89.5	16.4	11.8
	48.8	48.7	48.3±3.7	50.1±3.4	98.9	102.9	7.6	6.8
	19.8	19.6	20.5±2.8	24.5±3.9	103.7	125.2	13.7	15.9
THC	787.4	787.4	748.2±145.6	963.8±128.8	95.0	122.4	19.5	13.4
	495.0	495.0	454.9±103.3	461.3±29.3	91.9	93.2	22.7	6.3
	99.0	99.0	83.4±19.3	98.3±9.7	84.3	99.3	23.9	9.9
	49.8	49.8	41.3±7.6	45.4±11.7	82.9	91.1	18.3	25.7
THC-COOH	787.4	787.4	802.7±114.1	775.4±88.5	101.9	98.5	14.2	11.4
	495.0	495.0	510.9±52.2	430.6±50.6	103.2	87.0	10.2	11.8
	99.0	99.0	102.7±11.8	76.4±10.3	103.8	77.2	11.4	13.4
	49.8	49.8	55.9±2.7	38.3±4.3	112.3	76.9	4.8	11.2

Determination of LODs and LOQs

The LODs and LOQs of different drugs-of-abuse were determined experimentally with the use of spiked urine and oral fluid samples at low concentration. The smallest measurable signals (x_L) for each targeted analyte were firstly determined by measuring the signal obtained from blank samples. The calculation of x_L of each targeted analyte was described in the experimental section. The LOD and LOQ of an analyte were then defined as the concentrations of the spiked samples that can give signal larger than x_L with the factor (k) equal to 3 and 10 respectively. An example for the determination of blank urine signals for cocaine is shown in Figure 3-5. The MRM signals for the detection of 0.5 ng/mL cocaine in urine using SPME-ESI-MS are shown in Figure 3-5b. The averaged signal (relative peak area,

6 experiments and 18 measurements) at that concentration was 0.0045, which was higher than the x_L value for detection of cocaine in urine (0.0039) and the averaged ion ratio was 0.30 which was within the range for detection of cocaine (0.19 – 0.31). Therefore, the LOD of cocaine in urine was determined as 0.5 ng/mL.

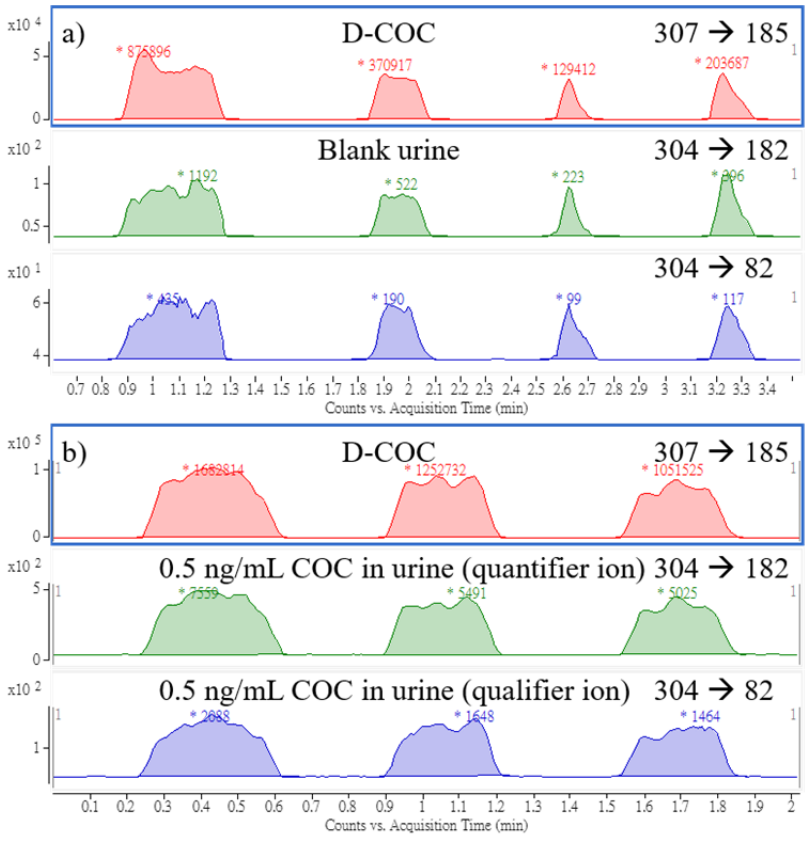

Figure 3-5. MRM results for the detection of cocaine of (a) blank urine and (b) 0.5 ng/mL of cocaine in urine using SPME-ESI-MS.

The LODs and LOQs obtained and the cut-off levels of international standards of the targeted analytes is listed in Table 3-3. The LODs obtained using SPME-ESI-MS were greatly

improved compared with those values obtained using WT-ESI-MS. For the detection of KET, Nor-K, MA, MDMA and COC, which were already good enough for real analysis, there were 2 – 25 times improvement. For the detection of BEN, HER, 6-MAM, MOR, THC and THC-COOH, the improvements were very obvious, from barely or not detectable to clearly detected even at low concentrations. The LODs of most of the drugs obtained using SPME-ESI-MS could fulfill the cut-off levels of international standards except for the detection of THC in oral fluid.

The improvements on the detection were due to the enrichment of targeted analytes onto the C18 SPME tip. C18 SPME tips also possess no porous structures and would not trap the targeted analytes onto the surface that may reduce the sensitivity of detection of the analytes. Moreover, the background signals generated by the SPME tip itself was very low, thus the resultant x_L values were also very low, which benefited the determination of LODs of the targeted analytes. However, the extraction and ionization efficiency for the detection of THC were still not good enough.

Table 3-3. LODs and LOQs obtained of the targeted analytes using SPME-ESI-MS and recommended cut-off values of various drugs in urine and oral fluid.

Compound	LOD (ng/mL)		LOQ (ng/mL)		SAMHSA cut-off (ng/mL)		EWDTS cut-off (ng/mL)		DRUID cut-off (ng/mL)
	Urine	O.F.	Urine	O.F.	Urine	O.F.	Urine	O.F.	O.F.
KET	10	5	10	10	N.A.	N.A.	N.A.	N.A.	N.A.
Nor-K	10	2	10	5	N.A.	N.A.	N.A.	N.A.	N.A.
MA	2	2	10	5	250	15	200	15	410
MDMA	2	2	10	10	250	15	200	15	270
COC	0.5	0.5	1	5	N.A.	8	N.A.	8.	170
BEN	10	8	15.6	15.6	100	8	100	8	95
HER	1	1	5	5	N.A.	N.A.	N.A.	N.A.	N.A.
6-MAM	1	1	5	5	10	2	10	2	16
MOR	5	5	10	10	2000	15	300	15	95
THC	50	50	125	62.5	N.A.	2	N.A.	2	27
THC-COOH	15	15	31.3	50	15	N.A.	15	N.A.	N.A.

3.3 Analysis of Medichem urine samples using SPME-ESI-MS

The results obtained and discussed in the previous sections were relied on the tested samples containing only same types of drugs. However, it is possible that the samples contained more than one types of drugs, if the drug abusers consumed different drugs simultaneously. The present of multiple analytes with different structure at the same sample may affect the analysis results as some of the drugs may be extracted and ionized more effectively than other drugs, which caused the signal suppression of the weakly ionized analytes. Three commercially available samples from Medichem which contained 29 drugs-of-abuse at different concentrations in human urine, were used to test the ability of SPME-ESI-MS for analyzing complex analyte mixture simultaneously. Medichem urine sample are the reference materials for forensic chemistry and used as same as the patient samples. The protocol used for handling the Medichem samples was the same as the protocol previously developed, but the extraction time was increased to 15 min as 5 or 10 min extractions were not enough to obtain stable signals for the analysis of such complex samples. The SPME-ESI-MS analysis of Medichem Basis-line U sample (contains no drugs-of-abuse) showed negative results (i.e. signals observed $< x_L$) for all targeted analytes (Figure 3-6), which were consistent with the manufacturer manual, as no false positive result was observed.

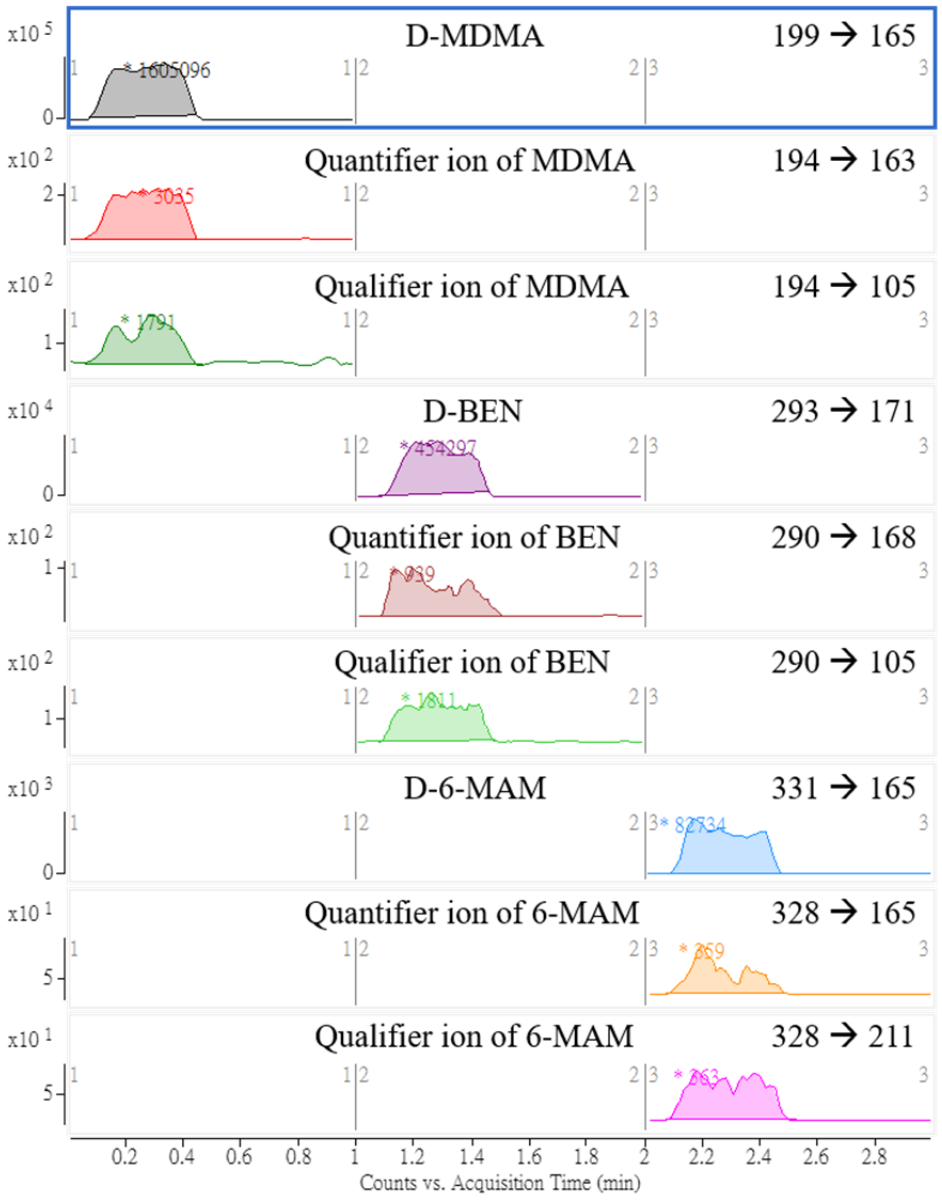

Figure 3-6. MRM signals for detection of MDMA, BEN and 6-MAM in the Medichem Basis-line U sample using SPME-ESI-MS.

Twenty-nine different drugs-of-abuse were contained in the samples of Medichem WDT confirm U -25% and +25%. The concentrations of each drugs-of-abuse varied, which were 25% lower and 25% higher than the EWDTS cut-off levels of that abuse-of-abuse. The

Medichem samples were used to investigate whether the established method could detect the drugs-of-abuse according to the EWDTS requirements. Respective results for analyzing the Medichem WDT confirm U +25% sample are shown in Figure 3-7. The targeted analytes that were present in both of the Medichem WDT confirm U -25% and +25% samples with the concentrations higher than the LODs of SPME-ESI-MS which could give positive results in the analysis, except for the detection of THC-COOH. Similarly, for the analytes that should give negative results, the SPME-ESI-MS analysis also gave negative results. The results for the quantitation of targeted analytes in the samples are listed in Table 3-4. The results were within reasonable range except serious under-estimation was observed for the quantitation of morphine, which the accuracy was less than 50% for both samples. Overall, no false positive result was obtained for the analysis of all analytes. False negative results were obtained only for the detection of THC-COOH. In addition, poor accuracy was observed for the quantitation of morphine. However, the results discussed previously showed that both morphine and THC-COOH could be detected at low concentrations when only that drugs and its metabolites were presented in the samples. The abnormal results obtained from Medichem samples may due to the relatively poorer ionization efficiency and extraction efficiency of morphine and THC-COOH when compared with other targeted analytes. The ionization of morphine and THC-COOH were suppressed by other easily ionized analytes in the complex samples.

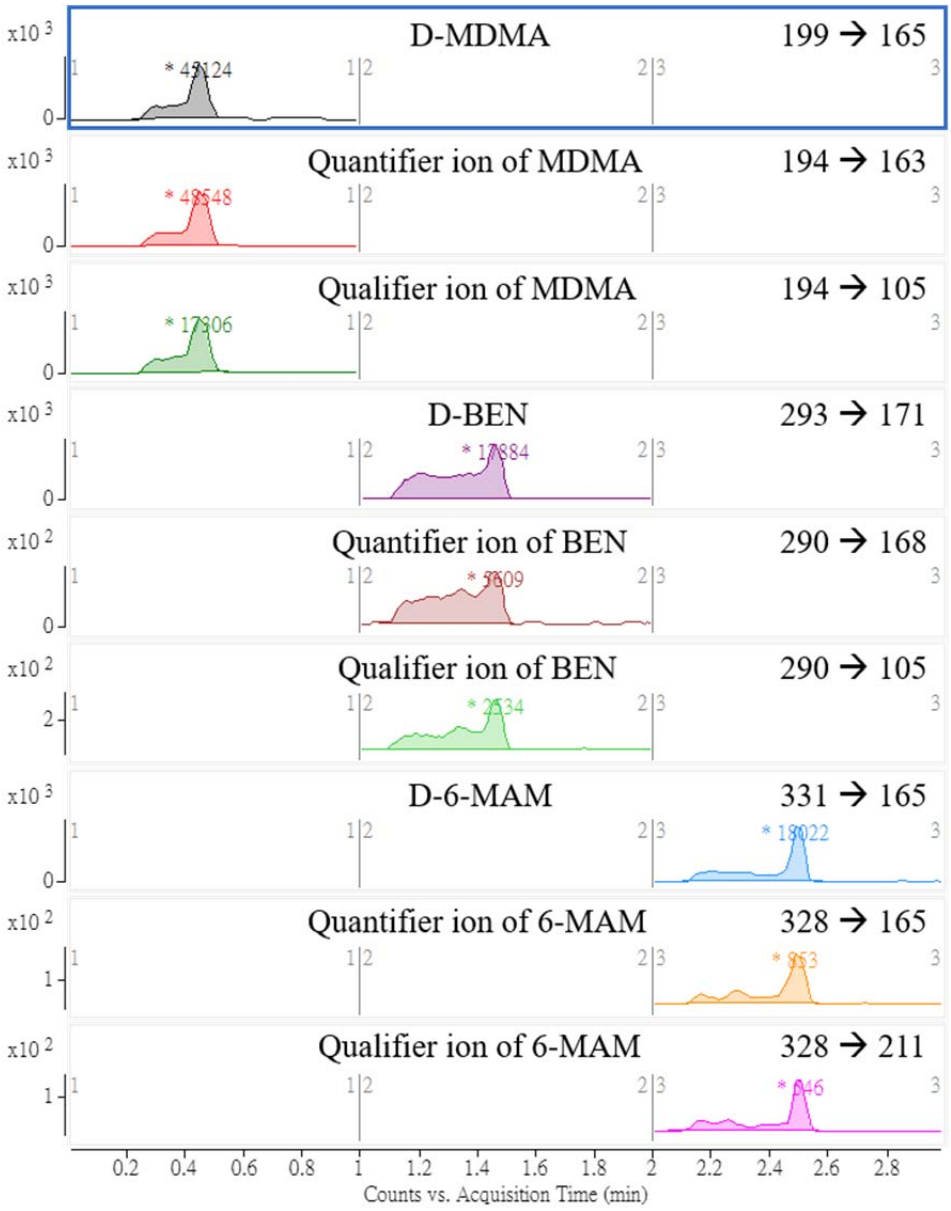

Figure 3-7. MRM signals for detection of MDMA, BEN and 6-MAM in the Medichem WDT confirm U +25% sample using SPME-ESI-MS.

Table 3-4. Results of quantitation for the targeted analytes in the Medichem samples using SPME-ESI-MS.

Compound	Medichem WDT confirm U -25%			Medichem WDT confirm U +25%		
	Actual conc. (ng/mL)	Measured conc. (ng/mL)	Accuracy (%)	Actual conc. (ng/mL)	Measured conc. (ng/mL)	Accuracy (%)
KET	N.D.	N.D.	---	N.D.	N.D.	---
Nor-K	N.D.	N.D.	---	N.D.	N.D.	---
MA	151.3	189.6	125.3	251.3	278.9	115.5
MDMA	143.5	111.2	77.5	241.5	182.0	72.4
COC	N.D.	N.D.	---	N.D.	N.D.	---
BEN	114.7	86.2	75.2	189.2	149.7	79.1
HER	N.D.	N.D.	---	N.D.	N.D.	---
6-MAM	7.9	8.0	101.0	11.9	10.0	84.3
MOR	225.9	107.5	47.6	361.2	153.3	42.4
THC	N.D.	N.D.	---	N.D.	N.D.	---
THC-COOH	10	N.D.	---	17.2	N.D.	---

N.D. = Not detectable (i.e. lower than the LODs)

3.4 Optimization of the SPME-p-GC-MS protocol

Optimization of the analyte extraction

As discussed in the previous sections, the sensitivity of the detection is highly dependent on the amount of analytes enriched on the SPME tip. Four parameters including selection of the SPME tip coatings, extraction methods, extraction time and addition of salt were optimized for the extraction of each targeted analytes.

There are five different SPME coatings including PDMS, PDMS/DVB, PA, CAR/PDMS and DVB/CAR/PDMS, available for GC analysis. All the above five SPME tips were tested for the extraction of all the targeted drugs in urine (1000 ng/mL). Only two drugs, ketamine and THC could be extracted and detected by SPME-p-GC-MS as shown in Figure 3-8. Ketamine gave the major fragments ion at *m/z* = 180 (highest intensity), 209 and 166, while THC gave the major fragments ion at *m/z* = 299 (highest intensity), 314 and 213. The fragment ions observed for the detection of ketamine and THC were consistent with that recorded in NIST database. However, no observable fragments ions for the detection of Nor-K, MA, MDMA, COC, BEN, HER, 6-MAM, MOR and THC-COOH were found.

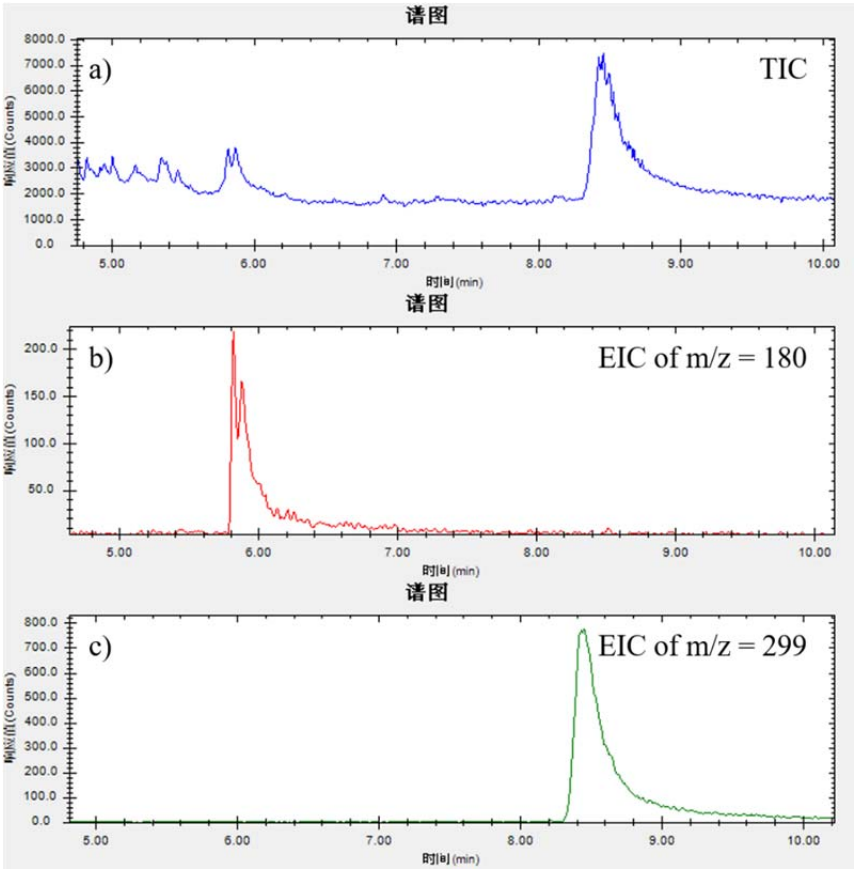

Figure 3-8. SPME-p-GC-MS results of analysis of all the targeted analytes in urine (1000 ng/mL) simultaneously using DVB/PDMS SPME tip. (a) Total ion chromatogram (TIC) and extracted ion chromatograms (EIC) of (b) *m/z* = 180 for the detection of ketamine and (c) *m/z* = 299 for the detection of THC respectively.

The detection of ketamine and THC in urine with lower concentrations were further investigated but only THC could give observable signals. Therefore, the extraction of THC was further optimized. The results of extraction of 1000 ng/mL THC in urine using different SPME tips are shown in Figure 3-9. The results showed PDMS/DVB, PDMS and PA SPME tips were able to extract THC in urine, among the materials, PDMS/DVB could give the

strongest signal and selected to use in further studies.

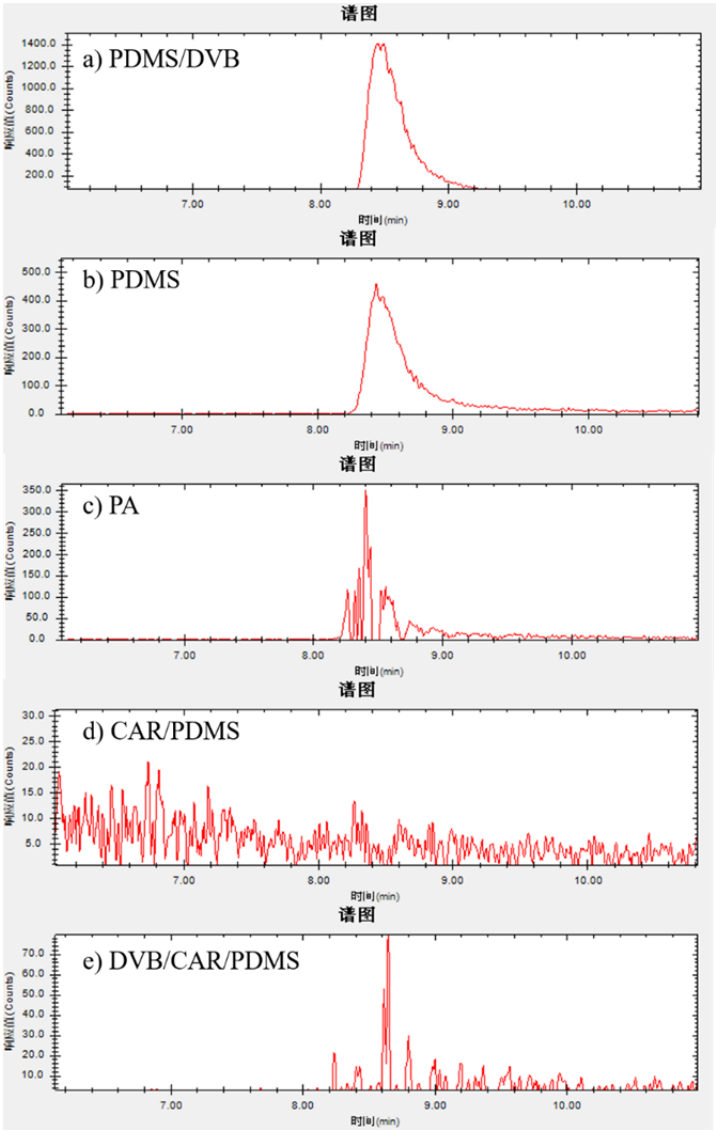

Figure 3-9. EIC of the detection of THC in urine using (a) PDMS/DVB, (b) PDMS, (c) PA, (d) CAR/PDMS and (e) DVB/CAR/PDMS SPME tip for the extraction respectively.

There are two extraction methods commonly applied for SPME, which are direct immersion (DI) and headspace (HS) extraction. The SPME tips are directly immersed into the liquid samples to extract the analytes in DI method while the SPME tips are placed above the liquid samples for the analyte extraction in HS method. HS extraction is more suitable for dirty samples as less interferences are extracted.[21] However, only analytes with relatively high volatility can be extracted by HS extraction, and addition of 25% NaCl and gentle heating are generally required to improve HS extraction. In this study, different SPME tips, additional of 25% NaCl and gentle heating up to 70^{o}C were tried for HS extraction of all the targeted analytes. However, no signals were observed for any targeted analytes using HS extraction. The analytes might tend to retain in aqueous samples rather than evaporate out. On the other hand, signals from ketamine and THC could be observed in immersion extraction and the addition of 25% NaCl showed no effect on the extraction efficiency. Therefore, immersion extraction without addition of NaCl was used in this study.

Finally, the extraction time of SPME method from 1 min to 40 min was tested. Similar to that of SPME-ESI-MS method, were the longer the extraction time, the stronger the signals could be obtained. However, to balance the extraction time with the extraction efficiency, 15 min extraction was selected in this study.

Chemical derivatization of drugs-of-abuse

Chemical derivatization is a common method used to enhance the sensitivity of GC-MS analysis. The targeted analytes are converted to more volatile species after reacting with derivatizing agents. Drugs-of-abuse often processes active hydrogens such as COOH, OH and NH, thus tend to form intermolecular hydrogen bonds or interact with solvent, which reduce the volatility of the compounds.[26] For this reason, there are a number of derivatizing reagents such as hexyl-chloroformate,[27] MSTFA[20] and PFPA[28] reported for derivatizing drugs-of-abuse prior to GC-MS analysis. In conventional derivatization methods, the drugs-of-abuse are firstly extracted from the samples, followed by evaporation of the extraction solvent. Finally, the derivatizing reagents are added into the dried residues for chemical derivatization.[28] However, the conventional procedures are time-consuming which is not suitable for rapid analysis. On-SPME-tip derivatization methods have been reported in some literatures such as Musshoff et al. (2002) and Merola et al. (2010).[20, 29] The on-SPME-tip derivatization methods allow reactions after SPME which can save the time for air-drying and the re-extraction of derivatized analytes using SPME. Two derivatizing reagents, MSTFA and PFPA were tried to react with the extracted drugs-of-abuse on SPME tips. Different SPME tips including PDMS, PDMS/DVB, PA, CAR/PDMS and DVB/CAR/PDMS were tried. However, no responding signals for the derivatized drugs-of-abuse could be observed. Also, no signals could be observed even when longer

extraction time and reaction time (up to 30 min) and higher reaction temperature (up to 90^{o}C) were used.

The poor results obtained even after derivatization might due to the poor extraction efficiency of drugs-of-abuse in aqueous solution using SPME tips. Most of the drugs-of-abuse possessed active hydrogens and tended to retain in aqueous solution rather than evaporate out. As the analytes were hardly extracted using HS extraction, the sensitivity of the portable instrument might not be good enough for the detection of analytes even after derivatization. Another possible reason is the poor derivatization efficiency of on-SPME-tip derivatization. SPME tips are tiny when compared with the area of reaction vials, the reaction surface might be too little for proper derivatizing reactions, thus reduced the efficiency of the derivatization.

3.4 Detection and quantitation of drugs-of-abuse in urine and oral fluid using SPME-p-GC-MS

Detection of drugs-of-abuse

Only two drugs-of-abuse, ketamine and THC, could be detected using SPME-p-GC-MS method as shown in Figure 3-8. The major fragment ions of ketamine (*m/z* = 180) and THC (*m/z* = 299) were used to monitor the presences of the drugs in samples. The identities of the drugs could be further confirmed by comparing the mass spectra of the analytes and reference spectra in the NIST database as shown in Figure 3-10.

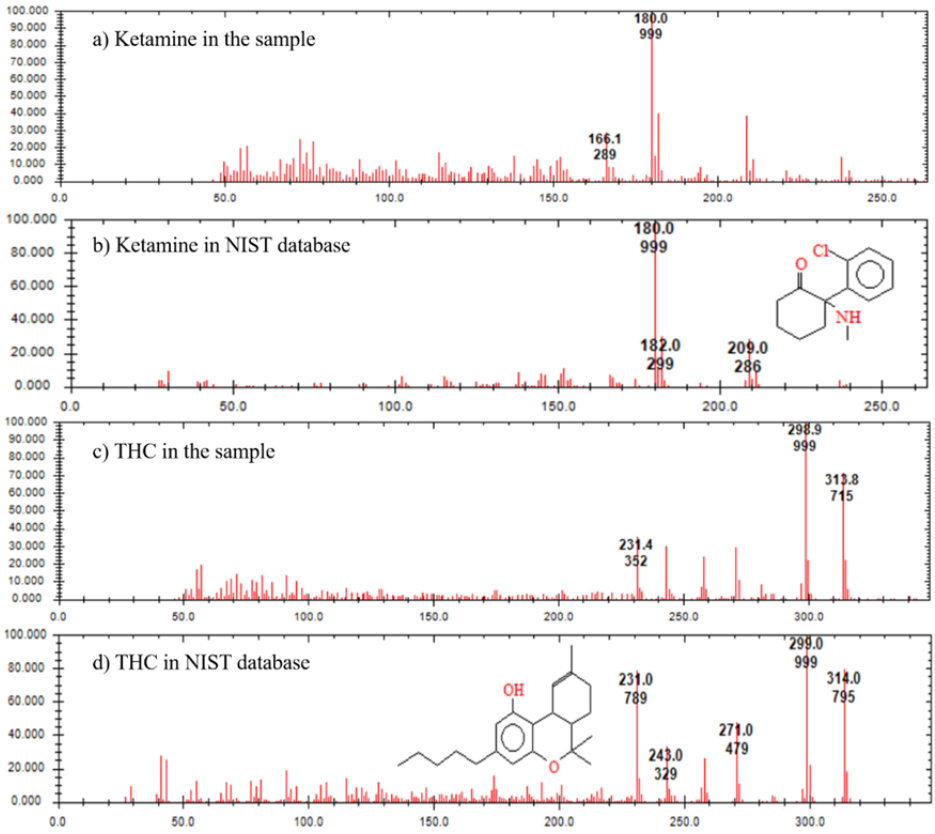

Figure 3-10. The portable GC-MS spectra of (a) ketamine in the sample, (b) ketamine in

NIST database, (c) THC in the sample and (d) THC in NIST database.

Quantitation of targeted analytes

Since ketamine could only be detected with very high concentrations and other drugs-of-abuse could not be detected using the SPME-p-GC-MS method, only the analytical performance of THC was tested in this project. The linear range was determined to be from 2000 ng/mL to 50 ng/mL and the calibration plot is shown in Figure 3-11. The calibration plot was constructed by the obtained from three different days. The precision (in term of R.S.D.) of the lowest two calibration points were poor, which were 31.1% and 43.0% for 250 ng/mL and 50 ng/mL respectively. The results indicated the relatively high fluctuation of the experimental conditions of the portable instrument. The accuracy and precision for the quantitation at low, middle and high levels of THC were generally better than 80% and within 20% as shown in Table 3-5, respectively.

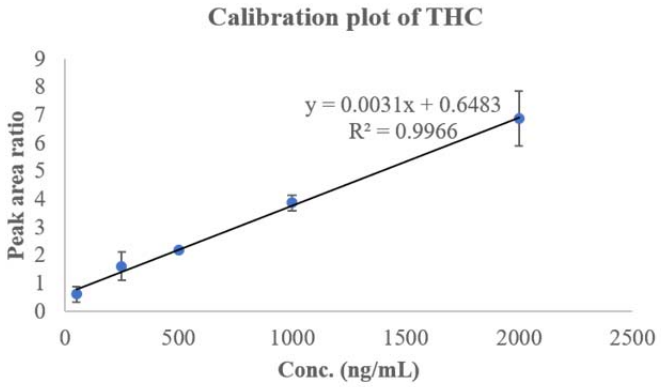

Figure 3-11. Calibration plots for the quantitation of THC in urine using SPME-p-GC-MS.

Table 3-5. Accuracy and precision for analysis of THC in urine using SPME-p-GC-MS.

Compound	Spiked quantity (ng/mL)	Determined quantity ± S.D. (ng/mL) (n=3)	Accuracy (%)	R.S.D (%)
THC	1574.8	1368.8 ± 65.9	86.9	4.8
	787.4	638.5 ± 5.3	81.1	0.8
	78.7	69.6 ± 15.8	88.4	22.7

Determination of LODs and LOQs

The EIC of the major fragment ion of THC at different concentrations are shown in Figure 3-12. The LOD (based on S/N > 3 for the strongest fragment ion) of THC was 20 ng/mL and the LOQ (based on S/N > 10 for the strongest fragment ion) of THC was 50 ng/mL. The LOD obtained was not good enough to fulfill the requirements of international standards and its metabolite, THC-COOH, could not be detected. SPME-p-GC-MS method might be good enough for analysis of some of the real-life samples only.

The overall performance of the portable GC-MS instrument for analysis of drugs-of-abuse in urine and oral fluid is not so satisfactory. There may be three major reasons for the poor performance: i) Most of the targeted analytes are small and relatively non-volatile molecules, which are not suitable for direct GC-MS analysis. ii) Derivatization of the targeted analytes on the SPME tips was not so effective, probably because the derivatizing agents could not

react with the analytes on the SPME tips effectively, as the sizes of the SPME tips were relatively small. Changing derivatization method may help but it may take longer time and require additional devices and procedures. iii) The sensitivity of the used portable GC-MS instrument was not good enough for the detection.

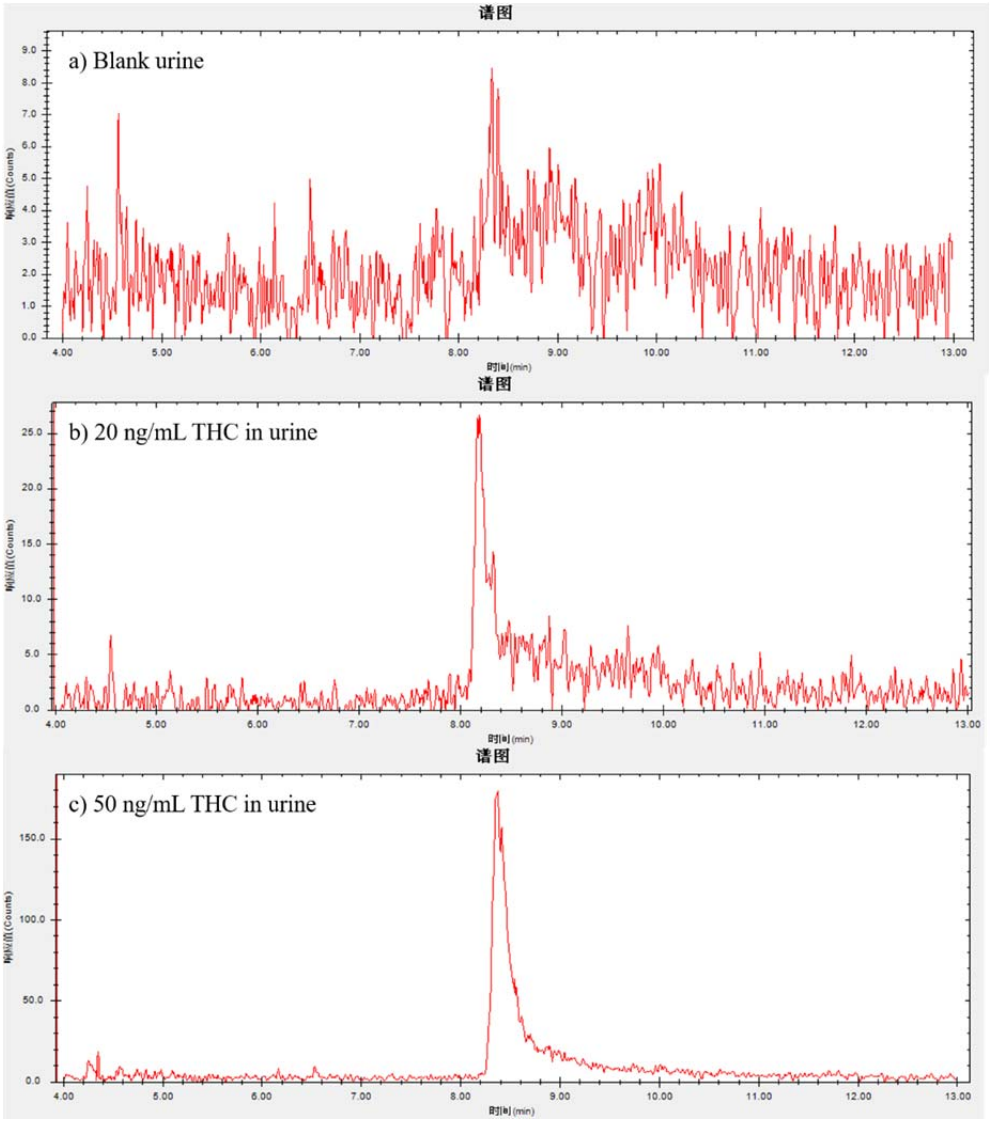

Figure 3-12. The EIC of major fragment ion of (a) blank urine, (b) 20 ng/mL THC in urine and (c) 50 ng/mL THC in urine.

4. Conclusions and further work

SPME-ESI-MS was developed for rapid and sensitive detection and quantitation of drugs-of-abuse in urine and oral fluid in this project. The sample preparation of SPME could be finished within reasonable time (5 – 10 min). Direct coupling of SPME with ESI-MS allowed analysis of analytes retained on SPME within minutes since no chromatographic separation was required. Compared with the WT-ESI-MS method that we developed in the previous project (BDF120020), the LODs of all the targeted drugs were much improved. The LODs of ketamine, methamphetamine, MDMA and cocaine were improved 2-20 times. For detection of heroin and THC, the improvements were much more obvious, from barely or not detectable to clearly detected even at low concentrations. The LODs of most of the drugs obtained using SPME-ESI-MS were able to fulfill the cut-off levels of international standards, except for detection of THC in oral fluid. In general, SPME-ESI-MS is simple and sensitive enough for analysis of drugs-of-abuse. Further improvements for detection of weakly ionized analytes such as morphine, THC and THC-COOH in complex samples could be considered, in order to better handle various possible problems in real cases. The use of other ionization techniques such as direct analysis in real time (DART) for detection of analytes that are weakly ionized in ESI may be useful. Development of SPME tips that can extract morphine, THC and THC-COOH more effectively would also improve detection of the weakly ionized

species.

The SPME-p-GC-MS method for analysis of drugs-of-abuse was also investigated in this project. After optimization of the experimental conditions, only signals from ketamine and THC could be observed using the potable instrument. Ketamine could be detected at high concentration (1 μg/mL) and the LOD of THC was 20 ng/mL, suggesting that the method might be applicable in some cases. Relatively large amount of samples (1.5 mL) was required in SPME-p-GCMS method, and such volume may not be readily available for oral fluid samples. Dilution of oral fluid samples is a possible solution but it would reduce the sensitivity of the detection. Although the results indicated that further improvement was still needed for the SPME-p-GC-MS method before its application to analysis of real-life samples, the development of such a method is important for prosecution of drug-after-driving and drug abuse in recreational centers. Use of better portable instruments and other ionization methods such as electrospray ionization or direct analysis-in-real-time may improve the on-site drug analysis.

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