Full Report

Project title: Rapid Detection and Quantitation of Drugs-of-abuse in Urine and Oral Fluid

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1. Background

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 problem of drug abuse, chemical analysis laboratories are required to handle a large number of body fluid samples for law enforcement and healthcare purposes. To deal with the large number of samples and ensure the reliability of the analytical results, analysis of drugs-of-abuse is currently performed with a two-step strategy, preliminary screening followed by confirmatory analysis[1-5]. Preliminary screening for the presence of illicit drug residues in body fluids is commonly performed by antibody-based on-site screening devices and immunoassay methods[2, 3, 5-13]. However, these methods have a number of problems, including cross-reactivity[3, 5, 6, 10] and generation of false positive and false negative results[5, 6, 9-14]. Therefore, the positive samples screened out in preliminary screening are further subjected to confirmatory analysis with advanced analytical techniques, e.g., gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS)[2, 4-6, 8, 13, 15-29]. However, these techniques commonly require extensive sample preparation procedures for reduction of matrix interference and analyte enrichment that could be time-consuming and laborious. For these reasons, development of simple, rapid and reliable methods for drug analysis has been an important task in drug abuse control.

2. Objective

This project was to develop a rapid and reliable method for detection and quantitation of drugs-of-abuse in urine and oral fluid by making use of wooden-tip electrospray ionization mass spectrometry (WT-ESI-MS), a technique developed by our research group in 2011[30]. This technique is simple, rapid, and of high tolerance to impurities, allowing rapid analysis of target

analytes in complex mixtures with little sample preparation and no chromatographic separation. Typically, analysis of one raw biological samples could be completed within minutes[31]. With these desirable features, this technique could be potentially applied for rapid detection and quantitation of drug analytes in body fluids such as urine and oral liquid, as demonstrated for ketamine and its metabolite norketamine in our preliminary study[31]. In this project, the WT-ESI-MS technique was further optimized and applied for rapid analysis of six drugs commonly abused in Hong Kong, including ketamine, methylamphetamine, cocaine, MDMA, cannabis (detected as tetrahydrocannabinol (THC)) and heroin.

3. Methodology

3.1 Instrumental setup

All WT-ESI-MS experiments were performed on a Micromass (Waters) Quattro Ultima triple quadrupole mass spectrometer. The experimental setup and workflow of WT-ESI-MS are shown in Figure 1[30, 31]. A wooden toothpick purchased from supermarket is first sharpened and then mounted onto the capillary holder of the nano-ESI ion source. Sample solution is applied to the tip end by pipetting. Upon application of a high voltage to the wooden tip, spray ionization is induced and ion signals of the analytes are detected. With the above experimental procedure, analysis of one sample could be completed within one minute.

3.2 Detection and quantitation of drugs-of-abuse in urine and oral fluid

Sample preparation for quantitative analysis

For quantitative analysis, the raw urine and oral fluid samples spiked with analyte standards and internal standards were diluted three folds with methanol. An aliquot of 2 μL of each prepared

sample solution was applied onto a wooden tip and analyzed with multiple reaction monitoring (MRM) mode.

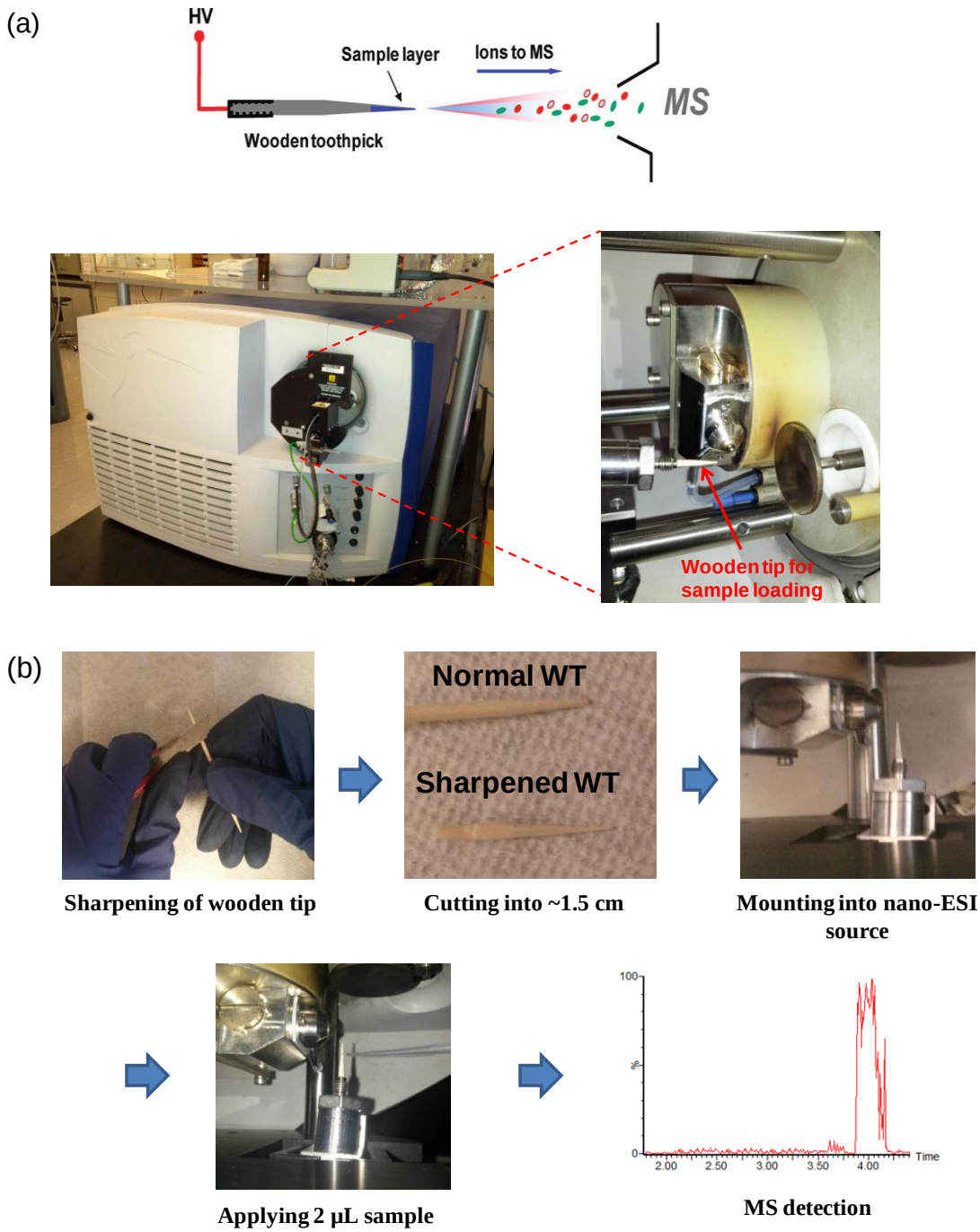

Figure 1. (a) Schematic diagram and photos for instrumental setup and (b) workflow for the WT-ESI-MS method.

Construction of calibration curves

The calibration curves for quantitation were constructed by averaging five sets of experimental data, while each set of data was obtained by applying samples containing different concentrations of the analytes and a fixed amount of the internal standard onto an individual wooden tip. Peak height was applied for constructing the calibration curves.

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

A blank sample was prepared by spiking only the internal standard to urine or oral fluid. The LOD and LOQ were determined by comparing the intensity (peak height) ratio of the analyte and internal standard between the spiked samples (i.e., the samples spiked with both analyte and the internal standard) and the blank sample $[(I_{analyte}/I_{IS})_{spiked}/(I_{analyte}/I_{IS})_{blank}]$ in order to compensate the chemical and electronic noises and variation in instrumental factors. The LOD and LOQ are defined as the quantity of analyte that could achieve a $[(I_{analyte}/I_{IS})_{spiked}/(I_{analyte}/I_{IS})_{blank}]$ value of three and ten, respectively.

Determination of accuracy and precision

At least three urine and oral fluid samples spiked with the analytes in low, medium, and high concentration ranges respectively were prepared. Each sample was analyzed for five times using an individual wooden tip, and the data obtained were averaged for comparison.

4. Results and Discussion

4.1 Optimization of experimental conditions

Different physical parameters, including configurations and orientation of the sample loading tip, the use of desolavation gas, and different materials of solid substrate, which could influence the analytical performance, i.e., sensitivity and reproducibility, were explored. We attempted to orient the sample loading tip orthogonal or parallel to the mass spectrometer inlet, introduce nitrogen desolvation gas to the tip, and apply different solid substrate materials, e.g., normal wooden tip, burnt wooden tip, nitric-acid treated wooden tip[32], aluminum foil[33], stainless steel needles[34, 35] and polymeric fibers[34], which were previously shown to be applicable for ESI analysis, as solid substrate for sample loading (Figure 2). In consideration of analytical performance, simplicity and economy, the orthogonal instrumental configuration (without desolvation gas) with normal wooden tip as sample loading solid substrate was selected and applied to the experiments in this project.

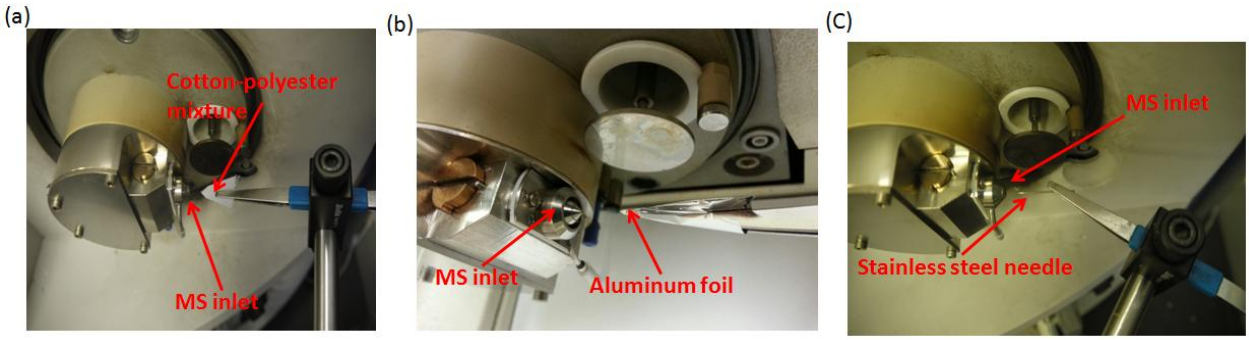

Figure 2. ESI source with (a) cotton-polyester mixture, (b) aluminum foil and (c) stainless steel needle as sample loading solid substrate.

The voltage and gas settings as well as the MRM conditions, i.e., collision energy, collision gas and cone voltage, for each drug analyte were optimized to achieve optimal sensitivity. The optimized MRM conditions for each drug analyte are shown in Table 1.

Table 1. MRM conditions of various drugs, metabolites and deuterium-labeled internal standards

Analyte	MRM Channel	Collision cell energy	Cone voltage
Ketamine	238 → 125	25	30
Nor-ketamine	224 → 125	20	30
Nor-ketamine-D4	228 → 129	20	30
Methamphetamine	150 → 91	15	30
Methamphetamine-D5	155 → 121	10	30
MDMA	194 → 163	8	30
MDMA-D5	199 → 165	10	30
Cocaine	304 → 182	15	30
Cocaine-D3	307 → 185	15	30
Benzoylecogonine	290 → 168	18	30
Benzoylecogonine-D3	293 → 171	18	30
THC	315 → 193	30	30
THC-D3	318 → 196	25	30
THC-COOH	343 → 299	25	30
THC-COOH-D9	352 → 308	25	30
Heroin	370 → 268	28	45
6-Monoacetylmorphine	328 → 165	35	40
6-Monoacetylmorphine-D3	331 → 165	32	35
Morphine	286 → 165	38	40

4.2 Establishment of experimental protocol

After optimization of various experimental conditions, an experimental protocol for analysis of abused drugs using WT-ESI-MS was established as follows:

1) Prepare samples by mixing the analyzed raw urine or oral fluid sample (spiked with internal standard) with methanol in a ratio of 1:2 (v/v).
2) Setup the nano-ESI source on the Micromass Quattro Ultima mass spectrometer.
3) Sharpen the tip end of a wooden tip to a diameter of ~ 0.2 mm, and cut the sharpen wooden tip to a length of ~ 1.5 cm.
4) Mount the sharpen wooden tip onto the capillary holder of the nano-ESI source. Adjust the position of the wooden tip until the tip end is at a position with perpendicular distance of ~10 mm and parallel distance of ~ 5 mm to the MS inlet.
5) Set capillary voltage to 3.5 kV and setup the MRM conditions, i.e., MRM channels, collision energy and cone voltage, according to Table 1.
6) Start data acquisition in MRM mode.
7) Apply 4 μL of methanol to wet the tip end of the wooden tip.
8) Apply 2 μL of the prepared sample to the tip end of the wooden tip for analysis of the sample.

4.3 Method validation

Construction of calibration curves

Representative data for construction of calibration curves is shown in Figure 3. Taking methamphetamine as an example, the intensities of MRM signals for analyte exhibited a positive correlation with the sample concentrations, while the signals for internal standard, which was at fixed concentration, did not significantly vary with different sample loadings (Figure 3a & b). Construction of a single calibration curve with typically five to seven data points could be completed in ten minutes or less. When plotting the intensity ratio of the analyte and internal standard with the concentration of spiked analyte, a calibration curve covering a concentration range of 25 – 5000 ng/ml (3 orders of magnitude) with linearity of $R^2 = 0.9996$ could be obtained (Figure 3c). The average standard deviation (average of standard deviation for different sample concentrations), as represented by the error bars, for data obtained in five experiments for construction of the calibration curve was 7.2%, indicating a high level of reproducibility of the present method.

The linear ranges of the present WT-ESI-MS method for the measurable drug analytes were generally in the concentration range of 10^1 to 10^3 ng/ml (3 orders of magnitude) (Table 2), which are comparable to conventional LC-MS and GC-MS methods and within the quantity ranges commonly found in drug abusers[36-43].

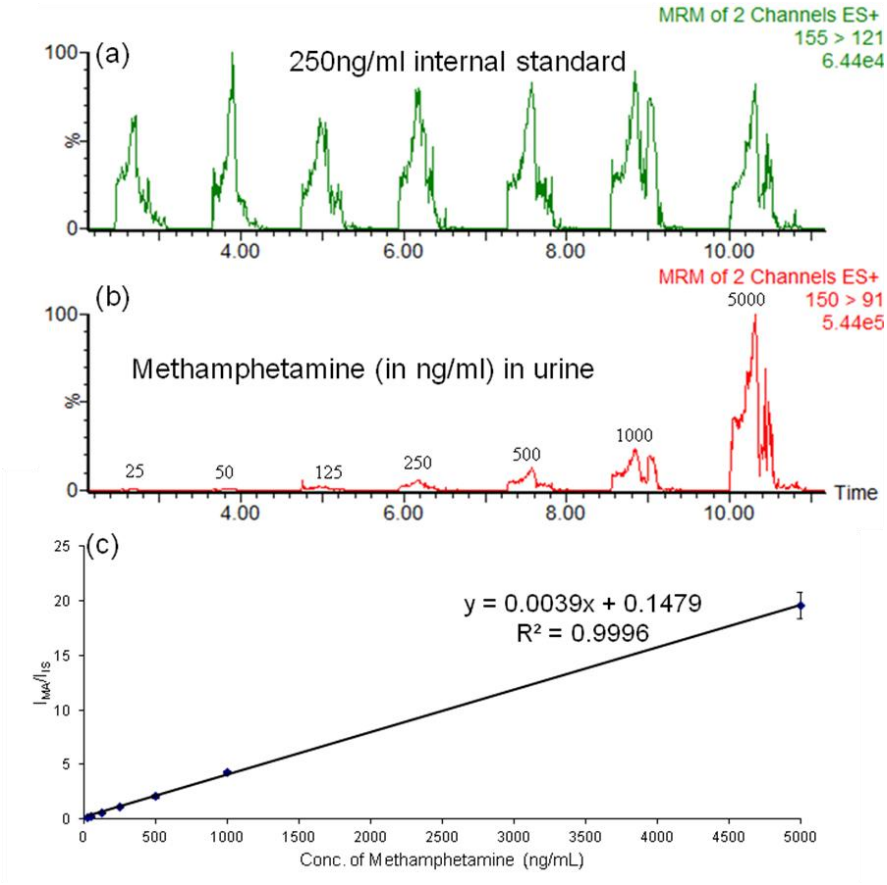

Figure 3. MRM signals for (a) 250 ng/ml of methamphetamine-D5 internal standard in urine and (b) different concentrations of methamphetamine in urine. (c) A calibration curve obtained for quantitation of methamphetamine in urine.

Determination of LOD and LOQ and their comparison with the recommended cut-off values

Representative spectral data for determination of LOD and LOQ are shown in Figure 4. With methamphetamine as an example, for the urine sample spiked with analyte, the signals of analyte could be much more clearly observed as compared to those of the blank urine sample, while the signals of the internal standard were similar to those of the blank sample. For methamphetamine, the LOD $[(I_{analyte}/I_{IS})_{spiked}/(I_{analyte}/I_{IS})_{blank} = 3]$ and LOQ $[(I_{analyte}/I_{IS})_{spiked}/(I_{analyte}/I_{IS})_{blank} = 10]$

determined were 12.5 ng/µl and 50 ng/µl, respectively (Figure 4). The LOD and LOQ for analysis of different drugs in urine and oral fluid are summarized in Table 2.

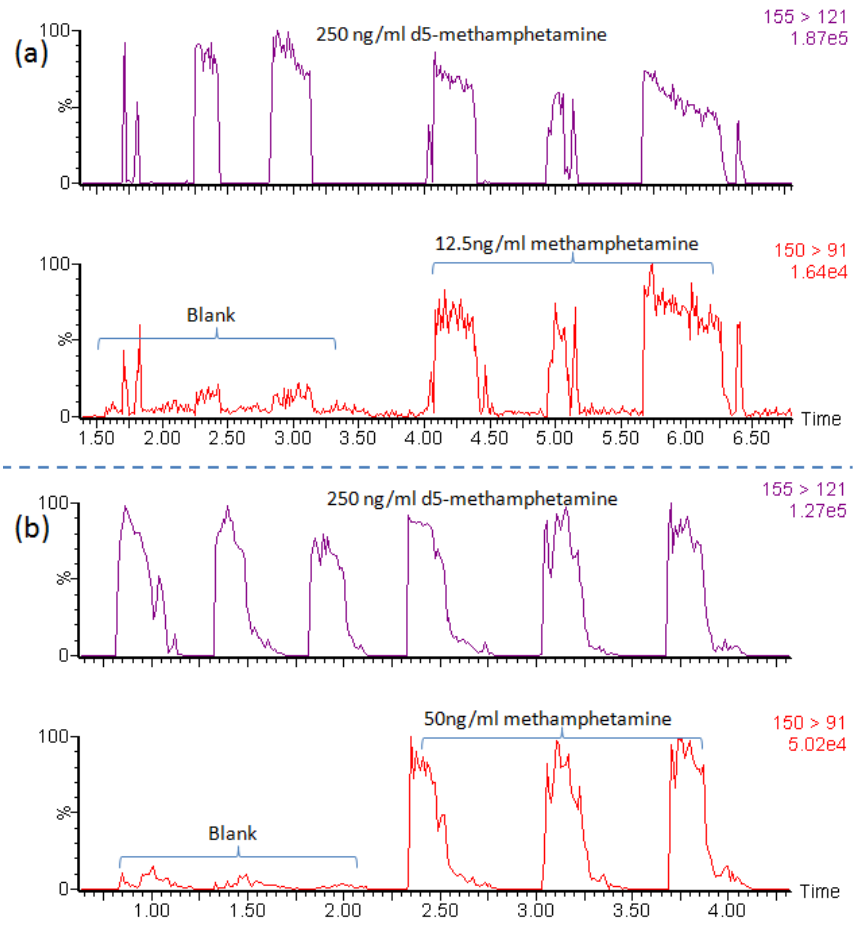

Figure 4. Spectral data for determination of LOD (a) and LOQ (b) for analysis of methamphetamine in oral fluid.

To evaluate the applicability of the present method in real applications, the LOD and LOQ for analysis of different drugs in urine and oral fluid are compared with the cut-off values recommended by guidelines produced by international authorities, including (1) Substance Abuse and Mental Health Services (SAMHSA)[44, 45], (2) European Workplace Drug Testing Society (EWDTS)[46, 47], and (3) Driving under the Influence of Drugs, Alcohol and Medicines

(DRUID) associated with European Union (EU)[48]. As this study aimed to apply the WT-ESI-MS as a one step method to confirm the presence of illicit drugs, the cut-off values for confirmatory analysis suggested by these guidelines were applied for comparison. Particularly for ketamine, the LOD and LOQ were compared with quantities of this drug commonly found in real case samples according to literatures, as no recommended cut-off value is available for comparison.

The LOD and LOQ determined for ketamine or norketamine in oral fluid or urine were 20 ng/ml and 50 ng/ml respectively. Compared to the amounts of these analytes commonly found in oral fluid and urine of drug abusers, which are in the range of 100 – 15,000 ng/ml [38], the LOD and LOQ achieved by the WT-ESI-MS were good enough for analysis of real samples. Our work on application of WT-ESI-MS method in analysis of ketamine and norketamine in urine and oral fluid has been published in *Analyst*, an international journal, as cover page[31] (Appendix I). For analysis of methamphetamine, the LOD and LOQ determined were generally within the cut-off levels recommended by the guidelines from SAMHSA, EWDTS, and DRUID, although the LOQ for analysis of oral fluid (50 ng/ml) was slightly higher than the cut-off values suggested by EWDTS (30 ng/ml). For analysis of MDMA, the LOD and LOQ obtained were generally lower than the cut-off values suggested in the three guidelines, yet LOQ for analysis of oral fluid (125 ng/ml) was slightly higher than the suggested cut-off values from SAMHSA (50 ng/ml) and EWDTS (30 ng/ml). These results revealed that the sensitivity of the present method is acceptable for analysis of methamphetamine and MDMA in real applications. For analysis of cocaine in oral fluid, the LOD and LOQ determined were within the cut-off level suggested by the guideline from DRUID. However, for analysis of benzoylecgonine, the major metabolite and marker of cocaine, as well as heroin and its metabolites, the LOD and LOQ determined were

higher than the suggested cut-off values from the three guidelines. For analysis of THC and its metabolites, THC-COOH, the sensitivity of detection was very poor and particularly no signal could be obtained for THC-COOH even at high concentrations. Further improvement in detection sensitivity is required for analysis of benzoylecgonine, THC, heroin, and their metabolites in real applications. The limited sensitivity could be due to the fact that the analytes tend to retain on the substrate surface and cannot spray out for detection, poor ionization efficiency and/or severe matrix interference. Development of more sensitive methods for analysis of drugs-of-abuse in body fluids is ongoing in our research group.

Accuracy and precision

The accuracy and precision for analysis of different drugs-of-abuse in urine and oral fluid are summarized in Table 3. For analysis of ketamine, norketamine, methamphetamine, MDMA, cocaine and benzoylecgonine, the precision was in general within 15% R.S.D as suggested in the method validation guideline from U.S. Food and Drug Administration (FDA)[49], except for methamphetamine in urine (17%) that was slightly higher than the recommended value. For analysis of heroin and its metabolites, the precision determined for analysis of oral fluids was satisfactory ($\leq$ 15%), yet for analysis of urine, the precision was found to be as high as 25%, mostly likely due to the relatively low absolute intensity. The accuracy of the present method for analysis of all analytes except for heroin and its metabolites was in the range of 75 – 122%, which is very close to the requirement of 80 – 120 % suggested by the method validation guideline from the FDA[49] and comparable to conventional LC-MS and GC-MS methods[8, 36, 37, 40, 43]. The accuracy and precision data for analysis of THC and its metabolites as well as morphine in urine were not available because of the poor sensitivity of detection.

Further confirmation of the presence of drugs by tandem mass spectrometry

The identities of drugs detected could be further confirmed by tandem mass spectrometry (MS/MS) analysis, in which the protonated ions of drugs were fragmented to produce fragment ions. The fragment ion patterns could be applied to confirm the identities of the drugs. For example, the identity of methamphetamine could be confirmed by the presence of fragment ions of *m/z* 91 and *m/z* 119 (Figure 5), or by comparison with the MS/MS spectrum of methamphetamine standard.

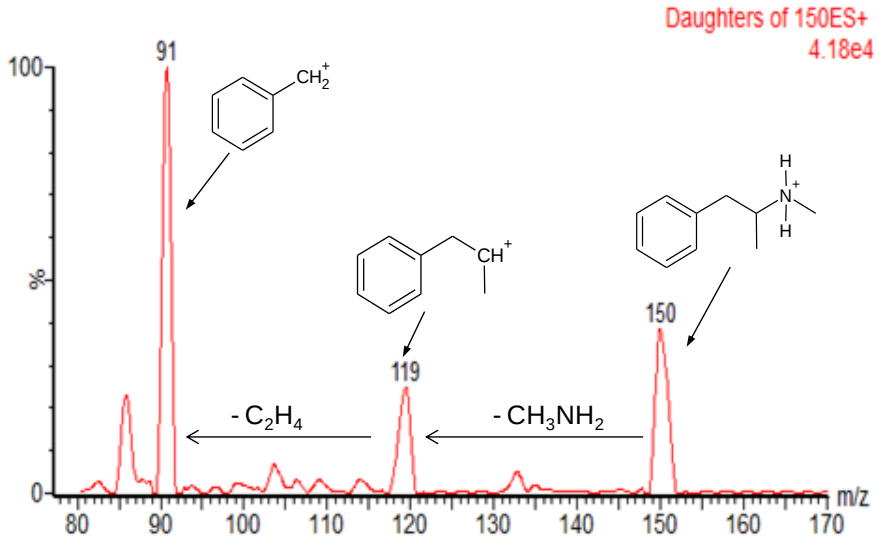

Figure 5. MS/MS spectra obtained for methanmphetamine (25 ng/ml in urine).

Table 2. LOD, LOQ, linear range and cut-off values for various drugs in urine and oral fluid.

Compound	LOD (ng/ml)		LOQ (ng/ml)		Linear range (ng/ml)		SAMHSA cut-off (ng/ml)		EWDTS cut-off (ng/ml)		DRUID cut-off (ng/ml)
	Urine	Oral Fluid	Urine	Oral Fluid	Urine	Oral Fluid	Urine	Oral Fluid	Urine	Oral Fluid	Oral Fluid
KET	20	20	50	50	50-5000	50-5000	N.A.	N.A.	N.A.	N.A.	N.A.
NKET	20	20	50	50	50-5000	50-5000	N.A.	N.A.	N.A.	N.A.	N.A.
mAMP	25	12.5	50	50	25-5000	25-5000	250	50	200	30	410
MDMA	50	50	250	125	50-5000	50-5000	250	50	200	30	270
COC	12.5	12.5	50	50	50-5000	25-5000	N.A.	N.A.	N.A.	N.A.	170
BZE	250	100	500	250	125-5000	50-5000	100	8	150	8	95
THC	40,000	40,000	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	2	27
THC-COOH	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	15	2	15	N.A.	N.A.
HRN	250	125	500	250	250-10000	125-5000	N.A.	N.A.	N.A.	N.A.	N.A.
6-MAM	500	125	1000	250	250-10000	125-10000	10	4	10	4	16
MOP	1000	500	10,000	10,000	500-10000	500-10000	2000	40	300	40	95

KET: ketamine; NKET: nor-ketamine; mAMP: methamphetamine; COC: cocaine; BZE: benzoylecgonine; HRN: heroin; 6-MAM: 6-monoacetylmorphine; MOP: morphine

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

Compound	Spiked Quantity (ng/ml)	Determined Quantity (ng/ml) (n=5)		Accuracy (%)		R.S.D (%)	
		Urine	Oral Fluid	Urine	Oral Fluid	Urine	Oral Fluid
Ketamine	100	107	84	107.4	84.1	9.3	13.1
	300	317	349	105.8	116.7	5.3	15.2
	600	587	690	97.8	115.0	11.9	11.3
	3000	3668	3162	122.3	105.4	6.8	1.8
Norketamine	100	95	82	95.7	81.8	10.5	7.3
	300	317	250	105.8	83.4	10.7	4.4
	600	601	638	100.2	106.3	4.3	7.8
	3000	3314	3470	110.5	115.7	5.2	11.2
Methamphetamine	100	105	114	105.7	114.3	17.0	6.3
	500	498	508	99.5	101.5	3.2	12.6
	1250	1105	1216	88.4	97.2	5.5	5.0
	2500	2536	2518	101.4	100.7	6.0	6.7
MDMA	100	112	117	112.1	117.1	6.8	4.6
	500	520	474	104.0	94.8	10.7	6.0
	1250	1186	1219	94.9	97.5	9.0	6.8
	2500	2492	2601	99.7	104	8.7	15.1
Cocaine	100	103	114	102.7	114.4	10.9	9.2
	500	510	489	102.1	97.8	9.0	11.8
	1250	1366	1296	109.3	103.7	5.8	12.8
	2500	2517	2561	100.7	102.4	4.6	12.5
Benzoylecgonine	500	432	461	86.3	92.1	8.6	13.2
	1250	1047	1331	83.8	106.4	7.9	10.3
	2500	2314	2657	92.6	106.3	9.6	7.9
Heroin	500	569	515	113.7	103.1	12.4	9.2
	1250	1349	977	107.9	78.2	18.3	7.7
	2500	2585	2346	103	93.8	15.9	7.4
6-Monoacetylmorphine	500	441	467	88.1	93.3	19.0	6.4
	1250	1220	1024	97.6	81.9	5.1	4.7
	2500	2678	2822	107.1	112.9	10.0	5.6
Morphine	500	N.A.	549	N.A.	109.9	N.A.	14.6
	1250	1343	940	107.4	75.2	25.5	6.3
	2500	2456	1880	98.2	75.2	16.3	9.3

5. Conclusions and further work

In this study, the application of the WT-ESI-MS method in rapid analysis of drugs-of-abuse in urine and oral fluid was investigated. Only little sample preparation and no chromatographic separation are involved in this method, allowing analysis of one body fluid sample within one minute. This method was demonstrated to have a broad linear range (3 orders of magnitude) and good accuracy and precision. The sensitivity of this method is generally adequate for rapid analysis of ketamine, norketamine, methylamphetamine and MDMA in urine and oral fluid as well as cocaine in oral fluid. For analysis of benzoylecgonine and the other two drugs, THC and heroin, and metabolites of these two drugs, further improvement in sensitivity is required to fulfill the analytical requirement recommended for reliable determination.

Our research group has been continuing to develop further advanced methods for analysis of drugs-of-abuse in body fluids. For example, we have recently developed a C18 pipette-tip ESI-MS technique, which has been demonstrated to be capable of rapid analysis of ketamine and norketamine in urine with LOD and LOQ 60 and 100 folds, respectively, lower than that of the WT-ESI-MS technique[50] (refer to appendix II, selected as cover story and featured paper by *Anal. Chim. Acta*). Furthermore, we are devoting to develop direct coupling of solid phase microextraction (SPME) with mass spectrometry for rapid analysis of drugs-of-abuse in urine and oral fluid with enhanced sensitivity. SPME, which makes use of a micro-tip coated with sorbent materials for rapid and selective extraction and enrichment of analytes in raw samples, is a commonly used technique for enhancing the sensitivity of chemical analysis[51, 52]. Our preliminary results encouragingly showed that SPME could be readily coupled with ESI-MS (SPME-ESI-MS) and the LOD of the SPME-ESI-MS method could be 2,000 times lower than

that of the WT-ESI-MS method and far below the cut-off values of most guidelines from international authorities. SPME could also be coupled with portable GC-MS, with potential for rapid on-site drug analysis. Research in these efforts is on-going in our group.

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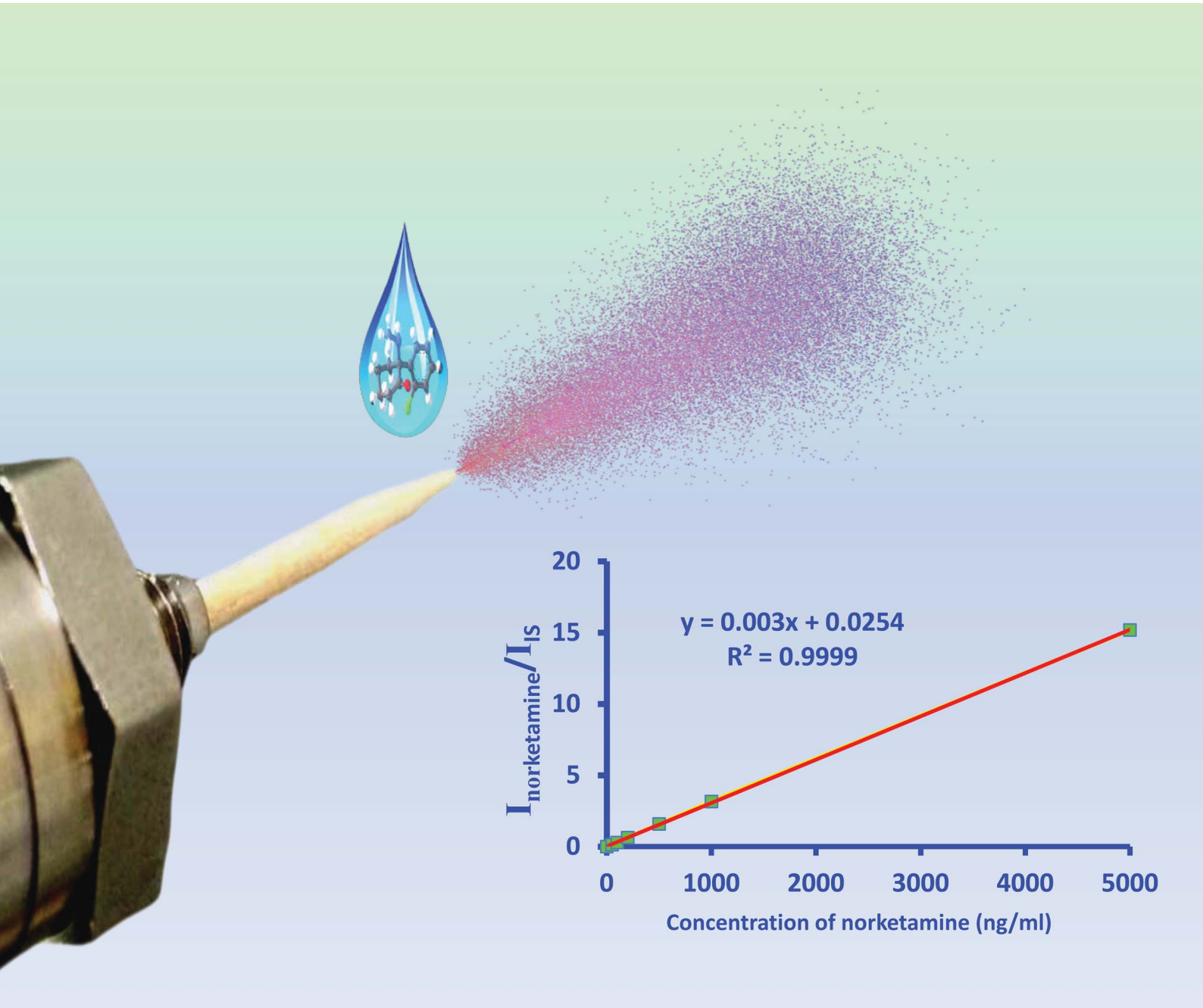

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Drug analysis is an indispensable task in controlling drug abuse, which is a serious problem worldwide nowadays. In this study, we report a simple and rapid approach for detection and quantitation of drugs-of-abuse in urine and oral fluid by wooden-tip electrospray ionization mass spectrometry (WT-ESI-MS). We demonstrated that ketamine, one of the most common abused drugs, and its major metabolite, norketamine, in raw urine and oral fluid could be readily detected and quantified by WT-ESI-MS with only little sample preparation and no chromatographic separation, and the analytical performances, including the linear range, accuracy, precision, LOD and LOQ, were well acceptable for analysis of real samples.

Drug abuse is a severe problem worldwide nowadays, and drug analysis is a critical step for drug abuse control. Identification of drug abuse is typically performed by determination of residues of drugs-of-abuse in body fluids (*e.g.*, urine, sweat, oral fluid and blood), nail and hair.[1–3] Analysis of body fluids is widely used because of the relatively high concentrations of drug and metabolite residues (in blood and urine), ease of collection (for oral fluid), and availability of relatively large sample volume (for urine).[2] Due to the prevalence of drug abuse, testing laboratories are required to handle a significant number of body fluid samples for law enforcement and rehabilitation purposes. To deal with the large number of samples and ensure the reliability of analytical results, a two-step strategy, preliminary screening followed by confirmatory analysis, is commonly applied.[4–8]

Preliminary screening for the presence of drug residues in body fluids is commonly performed on-site using commercially available on-site screening devices or in the laboratory using immunoassay techniques.[4–19] However, false positive and false negative results were potentially obtained by commonly used on-site screening devices and immunoassay methods.[8–10,15,17,20] The problem of cross-reactivity is also commonly encountered in these screening techniques, lowering the specificity of detection and increasing the potential of obtaining false positive and false negative results.[6,8,9,13,15–18] Moreover, preliminary screening typically cannot provide quantitative analysis of the samples.

Confirmatory analysis, which is to confirm the presence of drugs and determine the quantities of the drugs and their metabolites in the samples, is mainly performed by using techniques such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS).[4–6,8–12,15–18,20–29] Due to the high chemical complexity of body fluids, the GC-MS and LC-MS methods typically require sample pretreatments, *e.g.*, solid-phase extraction and liquid–liquid extraction, and chromatographic separation that are usually time-consuming.[4–6,8–12,15–18,20–29] Particularly for GC-MS, tedious derivatization of analytes is often required for effective vaporization and ionization of analytes.[22,23] The cost of consumables, such as consumable kits for extraction of samples and solvents for sample extraction and chromatographic separation, is also relatively high. Therefore, development of novel analytical methods that are simple, rapid, economical and reliable is highly beneficial to the field of drug analysis. For example, ambient mass spectrometric techniques, including desorption electrospray ionization (DESI) and desorption atmospheric pressure photoionization (DAPPI), have been attempted for rapid and direct analysis of drugs-of-abuse in urine.[30]

In this study, we report a simple and rapid method for measurements of drugs-of-abuse in urine and oral fluid by wooden-tip electrospray ionization mass spectrometry (WT-ESI-MS), a technique developed by us recently.[31] This technique has been demonstrated to be economical (only including wooden "toothpicks" as consumables and low consumption of solvents), easy-to-setup, readily compatible with different instruments, and applicable to analysis of a wide range of analytes.[31]

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Particularly, this technique is highly tolerant to impurities and matrices, allowing direct analysis of target analytes in complex mixtures with little sample preparation and no chromatographic separation.[32] With these desirable features and the high specificity of mass spectrometric detection, this technique could be applied as a rapid and reliable method for measurements of drug analytes in urine and oral liquid.

Rapid determination and quantitation of ketamine, one of the most common abused drugs, and its major metabolite, norketamine, in urine and oral fluid by WT-ESI-MS was demonstrated in this study. The WT-ESI technique was setup as described previously.[31] Briefly, a nano-ESI ion source was used for analysis on a triple-quadrupole mass spectrometer (Waters Quattro Ultima), and wooden toothpicks purchased from supermarkets were modified as described previously[31] and mounted onto the capillary holder of the ion source. Sample solution is applied to the tip end by pipetting. Upon application of a high voltage (3.5 kV in this study) to the wooden tip, spray ionization was induced and ion signals were obtained. The raw urine and oral fluid samples spiked with ketamine, norketamine, and internal standard d_4-norketamine were diluted three times with methanol. An aliquot of 2 μL of the prepared sample solution was applied onto a wooden-tip for analysis. The mass spectrometer was operated in selected-reaction monitoring (SRM) mode with selected reactions, m/z 238 → m/z 125, m/z 224 → m/z 125, and m/z 228 → m/z 129, for detection and quantitation of ketamine, norketamine, and d_4-norketamine, respectively. Other settings were similar to those in the previous study.[31]

The SRM chromatograms acquired by applying urine or oral fluid samples, which contained different concentrations of the analytes and a fixed concentration of the internal standard, onto wooden tips are shown in Fig. 1. Upon application of 2 μL of sample solution, distinct peaks with a time window of 20–40 s could be immediately observed in the SRM chromatograms. A positive correlation was observed between the peak intensity and the concentration of ketamine or norketamine (Fig. 1a and b). The peak intensity for the d_4-nonketamine internal standard did not vary significantly for different samples (Fig. 1c). The peak heights and peak areas of the SRM spectral peaks could be readily determined. Since no sample extraction and no chromatographic separation were required, analysis of such a single sample by WT-ESI-MS typically could be completed within one minute.

The calibration curves for quantitation were constructed by averaging five sets of experimental data, while each set of data was obtained by applying samples containing different concentrations of the analytes and a fixed amount of the internal standard onto an individual wooden tip. The internal standard was used to compensate variations in instrumental responses, which were mainly caused by use of different wooden tips and different sample loadings. The importance of the internal standard was also demonstrated in recent studies in quantitation of pharmaceuticals in blood spots using paper spray.[33,34] The peak height instead of the peak area was used for constructing the calibration curves and quantitation, as different extents of peak tailing of the analyte and internal standard signals might lead to poorer reproducibility of the data when the peak area was used for calculation.

The calibration curves obtained for ketamine and norketamine with d_4-norketamine as the internal standard are shown in Fig. 2. For both urine and oral fluid, the calibration curves obtained for both ketamine and norketamine exhibited excellent linearity (a R^2 value of at least 0.998) over the concentration range of 50–5000 ng mL^{-1} (Fig. 2). This linear range achieved is comparable to the GC-MS and LC-MS methods in previous

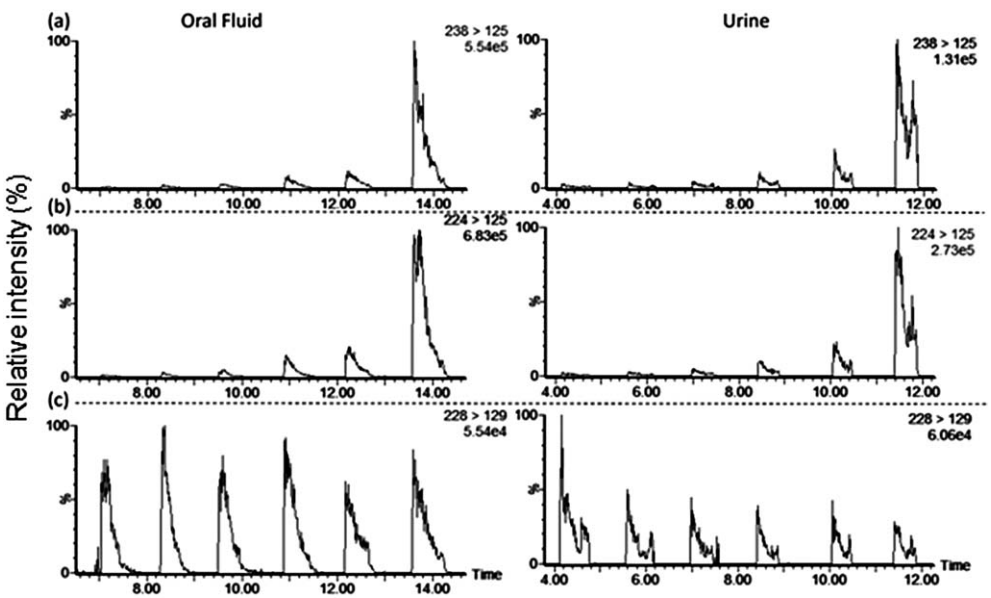

Fig. 1 SRM chromatograms obtained for 50–5000 ng ml^{-1} of (a) ketamine (m/z 238 → m/z 125) and (b) norketamine (m/z 224 → m/z 125) in oral fluid (left) and urine (right). The spectra peaks displayed are in the concentration sequence of 50, 100, 200, 500, 1000, and 5000 ng ml^{-1}. (c) SRM chromatograms obtained for 333 ng ml^{-1} of internal standard d_4-norketamine (m/z 228 → m/z 129) in oral fluid (left) and urine (right).

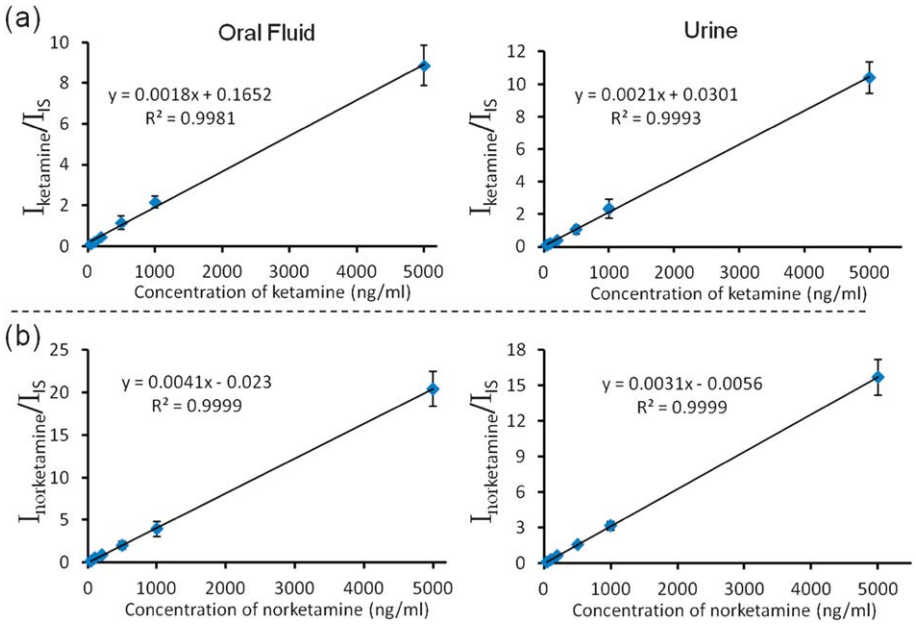

Fig. 2 Calibration curves obtained for (a) ketamine and (b) norketamine in oral fluid (left) and urine (right).

studies and covers the quantity of the two chemical components commonly found in urine and oral fluid samples from drug abusers.[5,6,21,23,24,26–28] These data demonstrated the applicability of the WT-ESI-MS method in quantitative analysis of real samples. The average relative standard deviation (RSD) of the data obtained from different wooden-tips ($n = 5$) was ~15%, revealing an acceptable level of reproducibility of the data obtained from different wooden tips, which might vary in physical morphology.

To investigate the accuracy and precision of the WT-ESI-MS method in direct quantitation of ketamine and norketamine, four urine and oral fluid samples spiked with the analytes in low, medium, and high concentration ranges respectively were analyzed. Each sample was analyzed five times using an individual wooden tip, and the data obtained were averaged for comparison. The accuracy and precision determined were in the range of 82–122% and 4.3–15.2%, respectively (Table 1), which were comparable to the LC-MS and GC-MS studies.[5,21–24,26–28]

For determination of the limit-of-detection (LOD) and limit-of-quantitation (LOQ) of the WT-ESI-MS method for measurements of the analytes, a blank sample was prepared by spiking the internal standard only to urine or oral fluid. Note that signals resulting from chemical and electronic noises were observed even upon application of a blank sample and the level of these noises might vary with different sample loadings and different wooden tips. To compensate such variations, the LOD and LOQ were determined by comparing the intensity (peak height) ratio of the analyte and internal standard of the spiked samples (*i.e.*, the samples spiked with both the analyte and the internal standard) with that of the blank sample, *i.e.*, $(I_{analyte}/I_{IS})_{spiked}/(I_{analyte}/I_{IS})_{blank}$. The LOD and LOQ are defined as the quantity of analyte that could achieve a $[(I_{analyte}/I_{IS})_{spiked}/(I_{analyte}/I_{IS})_{blank}]$ value of three and ten, respectively. The LOD and LOQ determined for ketamine or norketamine in oral fluid or urine were 20 ng ml^{-1} and 50 ng ml^{-1} (Fig. 3), respectively, which were suitable for analysis of real samples.[5,6,23,24,26]

Table 1 Experimental data for determination of accuracy and precision of the WT-ESI-MS method in quantitation of ketamine and norketamine in oral fluid (OF) and urine

		Determined quantity (ng ml^{-1}) ($n = 5$)		RSD (%)		Accuracy (%)	
Spiked quantity (ng ml^{-1})		Urine	OF	Urine	OF	Urine	OF
Ketamine	100	107 ± 10	84 ± 11	9.3	13.1	101	84
	300	317 ± 17	349 ± 53	5.3	15.2	106	116
	600	587 ± 70	690 ± 78	11.9	11.3	96	115
	3000	3668 ± 248	3162 ± 58	6.8	1.8	122	105
Norketamine	100	95 ± 10	82 ± 6	10.5	7.3	95	82
	300	317 ± 34	250 ± 11	10.7	4.4	106	83
	600	601 ± 26	638 ± 50	4.3	7.8	100	106
	3000	3314 ± 172	3470 ± 387	5.2	11.2	110	116

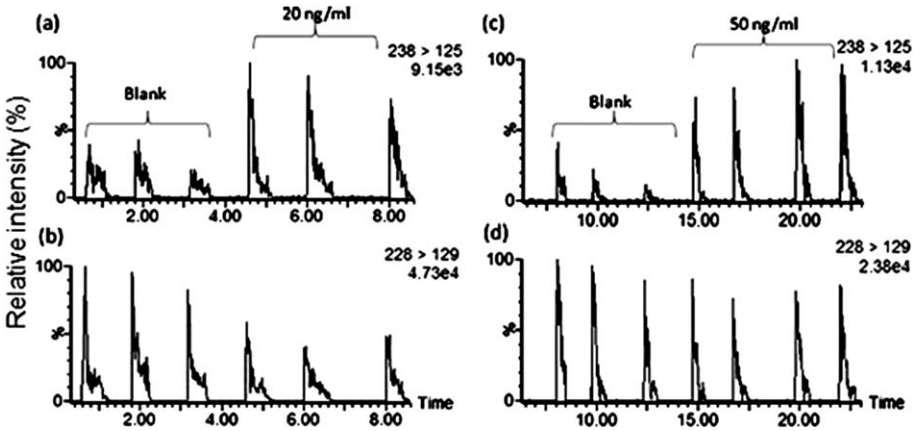

Fig. 3 (a and b) SRM chromatograms obtained for ketamine and internal standard d_4-norketamine respectively in oral fluid for determination of LOD. The LOD $[(I_{analyte}/I_{IS})_{spiked}/(I_{analyte}/I_{IS})_{blank}] = 3$) for detection of ketamine in oral fluid was determined to be 20 ng ml^{-1}. (c and d) SRM chromatograms obtained for ketamine and internal standard d_4-norketamine respectively in oral fluid for determination of LOQ. The LOQ $[(I_{analyte}/I_{IS})_{spiked}/(I_{analyte}/I_{IS})_{blank}] = 10$) for detection of ketamine in oral fluid was determined to be 50 ng ml^{-1}. Similar spectral results were obtained for ketamine in urine samples and norketamine in both oral fluid and urine samples for determination of LOD and LOQ.

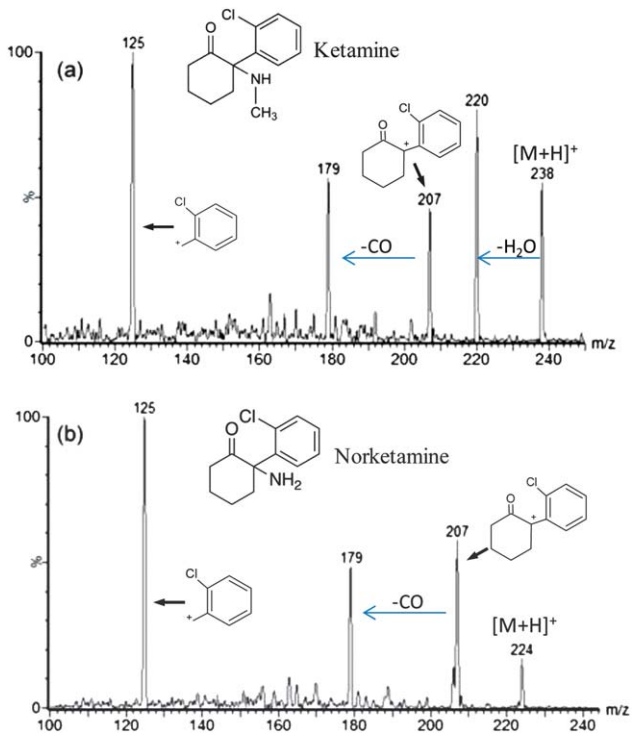

Fig. 4 Product ion mass spectra obtained for 50 ng ml^{-1} of (a) ketamine and (b) norketamine in oral fluid. Structures of ketamine and norketamine and potential structures of the major product ions are labeled in the spectra. Similar spectral results were obtained for urine samples.

The identities of the ketamine and norketamine analytes could be further confirmed by product ion scan experiments. As shown in Fig. 4, quality product ion mass spectra could be obtained for ketamine and norketamine at a low concentration range in oral fluid or urine using the WT-ESI-MS method, showing distinct peaks of fragment ions reported previously[24] and allowing reliable determination of the identities of the two drugs.

This study demonstrated that WT-ESI-MS could be applied as a rapid method for detection and quantitation of ketamine and norketamine in biological fluids. This method involves only little sample preparation and no chromatographic separation, significantly reducing the cost and time required for analysis. The identities of the drugs could be confirmed with product ion scan experiments, and the analytical performances, including the linear range, accuracy, precision, LOD and LOQ, of the method were well acceptable for analysis of real samples. Further investigation will be performed to further optimize this technique and establish it as a simple, rapid and reliable method for analysis of common drugs-of-abuse.

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Rapid analysis of raw solution samples by C18 pipette-tip electrospray ionization mass spectrometry

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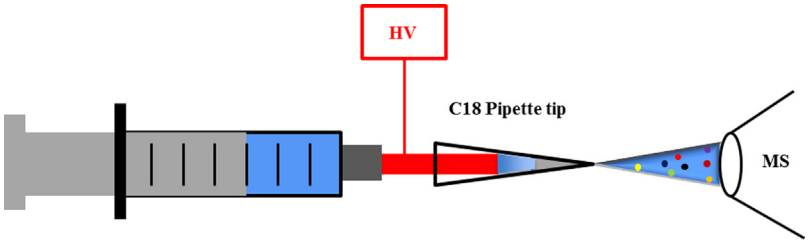
HIGHLIGHTS

- Combination of C18 pipette tips with syringe and syringe pump for rapid analysis of raw solution samples by mass spectrometry.
- Rapid analysis of protein solutions containing high contents of salts and detergents.
- Rapid quantitation of ketamine and norketamine in urine with very low limits of detection.

GRAPHICAL ABSTRACT



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ABSTRACT

A C18 pipette-tip electrospray ionization mass spectrometry technique was developed for rapid analysis of raw solution samples. In this technique, a C18 pipette tip was employed for rapid purification and enrichment of analytes in raw sample solutions. The adsorbed analytes were eluted by solvents supplied by a syringe and a syringe pump, and a high voltage was applied onto the syringe needle to induce electrospray ionization at the pipette tip end for mass spectrometric analysis. This technique is simple, easy to assemble, enables generation of stable and reproducible signals, and can be conveniently used for qualitative and quantitative analysis of raw solution samples. Analysis by the technique only involved simple sample preparation procedures followed by direct mass spectrometric detection, all of which could be completed within minutes, while the analytical performances of the technique, including the limit of detection, limit of quantitation, liner range, accuracy and precision, were comparable to those by conventional methods.

1. Introduction

Mass spectrometry (MS) is a powerful tool for qualitative and quantitative analysis of various analytes, and electrospray ionization (ESI) is a commonly used MS technique [1]. ESI-MS analysis of raw samples typically requires labor-intensive sample pretreatment, including extraction, enrichment, chromatographic separation, etc. [2–4]. In the past decade, great efforts have been made to enable direct analysis of raw samples by ESI-MS and various ambient ionization techniques have been developed [5–7].

In recent years, our group has devoted to develop new techniques to facilitate ESI-MS analysis [8–14]. Very recently, we developed a pipette-tip ESI-MS technique [14] for direct analysis of raw solid samples. Pipette-tip ESI-MS combines common pipette

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tips with syringe and syringe pump and allows on-line extraction and ionization of raw solid samples placed inside the pipette tips. We demonstrated that pipette-tip ESI-MS could be conveniently used for rapid analysis of various solid samples, particularly herbal powders, with stable, durable and reproducible signals.

In this study, the pipette tip used in the previous technique was replaced with a C18 pipette tip (Fig. 1) and the technique was further developed for analysis of raw solution samples. Pipette tip-based micro-extraction (TBME) has been well developed for sample desalting, purification and enrichment [2,15,16]. C18 pipette tip, i.e., pipette tip containing C18 sorbent, offers rapid sample preparation for mass spectrometric analysis, and its commercial product, i.e., ZipTip, has been commonly available. Inducing electrospray ionization directly from a pipette tip column has been attempted previously [17–19], typically involving insertion of a metal wire for connection of the high voltage [17,18] and use of an additional power supply [18]. As shown in Fig. 1, our current technique makes use of the metal syringe needle and solvents for delivery of the high voltage, rendering the device simple and easy to assemble. Moreover, in our technique, continuous and controllable supply of solvents by the syringe pump allows durable and reproducible signals and subsequently reliable quantitation of the analytes. After sample loading and clean-up, analyte molecules bound to C18 bed are eluted with the elution solvent and directly sprayed out for ESI-MS analysis with the application of a high voltage to the syringe needle. The present technique, termed as C18-pipette tip ESI-MS herein, involved typically 2–3 min of sample loading and cleanup procedures followed by direct ESI-MS detection, allowing analysis of a raw solution sample within 5 min. In this study, C18-pipette tip ESI-MS was employed to analyze protein solutions containing salts and detergents and quantitation of ketamine and norketamine in urine.

Salts and detergents are commonly present in protein samples [4,20]. Salts such as NaCl are widely used to mimic the physiological environment of organisms for in vitro protein research, while detergents such as sodium dodecyl sulfate (SDS) are commonly employed for protein isolation and solubilization, especially for membrane proteins that are usually of poor solubility [3,21]. Mass spectrometry is the method of choice for analysis of proteins, however, it is not compatible with salts and detergents. Removal of salts and detergents from protein samples is thus essential prior to mass spectrometric analysis and various methods have been employed [3,4,22,23]. In this study, C18 pipette-tip ESI-MS was attempted for analysis of protein solutions containing salts and detergents, in an effort to develop simple and rapid approaches for detection of proteins in the presence of salts and detergents.

Drug abuse, especially abuse of psychotropic drugs, is a serious problem worldwide. Ketamine is a commonly abused psychotropic drug to induce psychedelic effects to the abusers [24], and norketamine is the major metabolite of ketamine in human body [25,26]. Identification of ketamine abusers is critical for drug control, which usually relies on measurements of ketamine residue and norketamine in the abusers' urine, blood, nail and hair [27–30]. Urine is commonly chosen for the measurement due to the relatively high concentrations of ketamine and norketamine and large sample volume available. Quantitation of ketamine and norketamine in urine is usually performed using MS combined with gas chromatography (GC) or liquid chromatography (LC) [27,29,31–33]. In order to reduce interferences of matrices in the urine samples, extensive sample pretreatments are required before chromatographic separation and MS detection [27,29,31–33]. Development of simple, rapid and high-throughput methods are thus highly desirable for the growing analytical demands in beat drugs campaigns. Very recently, direct detection and quantitation of ketamine and norketamine in urine and oral fluid using wooden-tip ESI-MS was successfully developed by our group [13]. The achieved linear range, limit of detection (LOD) and limit of quantitation (LOQ) were 50–5000 ng mL^{-1}, 20 ng mL^{-1} and 50 ng mL^{-1} respectively for quantitation of both ketamine and norketamine in urine [13]. For analysis of various real samples, a linear range with lower concentrations and lower LOD and LOQ may be required [29] and a new method is thus expected. In this study, C18 pipette-tip ESI-MS was examined for rapid quantitation of ketamine and norketamine in urine.

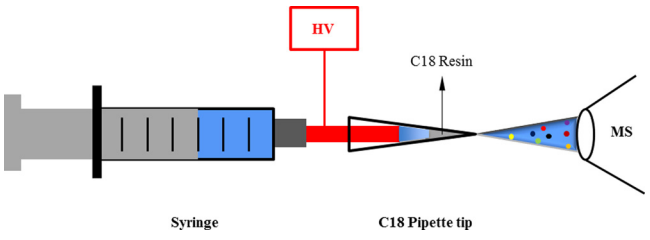

Fig. 1. The schematic diagram of C18 pipette-tip ESI-MS.

2. Experimental

2.1. Materials and chemicals

C18 pipette tips, i.e., 10 μL ZipTip containing 0.6 μL C18 resin, were purchased from Millipore (USA). The C18 resin was made of silica of 15 μm diameter and 200 Å pore size. Cytochrome c from equine heart, myoglobin from equine heart, α-lactalbumin from bovine milk and sodium dodecyl sulfate (SDS) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Urine was collected from a healthy volunteer. Water was prepared using a Milli-Q system (Millipore Laboratory, USA). All the other solvents were of HPLC grade and purchased from Tedia (Fairfeild, OH, USA).

2.2. Sample preparation

For the detection of proteins, 5 μM cytochrome c, myoglobin and α-lactalbumin were prepared in the solution of NaCl (1%) or SDS (0.1%). Then the protein solution was mixed with methanol containing 0.2% formic acid (FA) in a volume ratio of 1:1 for conventional ESI-MS analysis as comparison, or treated with C18 pipette tip for subsequent C18 pipette-tip ESI-MS analysis.

For quantitation of ketamine and norketamine, standard solutions of ketamine or norketamine were prepared with concentrations of 400, 100, 50, 10, 5, 2 μg mL^{-1} in methanol, and internal standard solution of d_4-norketamine was prepared with a concentration of 50 μg mL^{-1} in methanol. Urine samples containing ketamine and norketamine for quantitative analysis were prepared with the followed procedures: 1 mL urine was spiked with 1 μL ketamine and 1 μL norketamine standard solutions of various concentrations (2, 5, 10, 50, 100, 400 μg mL^{-1}), then 1 μL of the internal standard solution was added to each solution. Finally, a set of urine samples containing ketamine and norketamine with different concentrations (2, 5, 10, 50, 100, 400 ng mL^{-1}) and d_4-norketamine with a fixed concentration (50 ng mL^{-1}) were obtained.

Procedures for using C18 pipette tip for removal of salts, detergents and other interfering agents were similar to the protocol described in the Millipore C18 ZipTip manual. Typically 10 μL sample was used for sample loading each time, and for quantitation of ketamine and norketamine in urine, this sample loading procedure was repeated six times for enrichment of

analytes. The loaded samples were washed with water containing 0.1% formic acid for three times. After the washing procedure, the C18 pipette tip was combined with the syringe and syringe pump for ESI-MS analysis. Methanol/water/formic acid (50/50/0.1, v/v/v) was used as the extraction and spraying solvent if not specified otherwise.

2.3. Instrumentation and setup

As shown in Fig. 1, the blunt point needle (i.d. 410 μm and o.d. 720 μm) of a glass syringe (250 μL, Hamilton) was inserted into the C18 pipette tip, the distal end of which had an i.d. of 3.4 mm and an o.d. of 5.8 mm. A syringe pump (Harvard Pump 11 Plus, USA) was employed to supply solvents with a flow rate of 5 μL min^{-1}. A high voltage (typically 5.5 kV) was applied to the stainless steel syringe needle with a clip and conducted to the pipette tip end through the solvent to induce electrospray ionization. High voltage hazard: operators must not touch any conductive components connected with the high voltage, i.e., the stainless steel syringe needle and the high voltage connecting clip. Mass spectrometric measurements were performed in positive ion mode.

For analysis of the protein solutions, the C18 pipette-tip ESI ion source was coupled with a quadrupole time-of-flight (Q-ToF) mass spectrometer (QStar Pulsar, Applied Biosystems, USA). The protein signals typically lasted for about 30 s and the spectra were obtained by accumulating the signal periods. Analysis of the protein solutions with conventional ESI-MS approach was performed on the same mass spectrometer with the equipped ESI ion source. The mass spectrometer was operated with a curtain gas flow of 30 A.U., and the spray voltage (IS), first declustering potential (DP1), focusing potential (FP) and second declustering potential (DP2) were set to optimum values.

For quantitation of ketamine and norketamine in urine, the C18 pipette-tip ESI ion source was coupled with a triple quadruple mass spectrometer (Quattro Ultima, Waters, USA), with the pipette tip end located at a position with a perpendicular distance of 3.0 cm and parallel distance of 1.0 cm to the MS inlet. The capillary voltage, cone voltage and source temperature were set at 3.8 kV, 30 V and 80 °C respectively. The elution and spray solvent used was 80% methanol containing 1.0% formic acid, and the flow rate was 5 μL min^{-1}. The equipment was performed in positive selected reaction monitoring (SRM) mode to allow detection of target analytes with high specificity and sensitivity. The product ion mass spectra and the fragmentation patterns of ketamine and norketamine have been investigated previously [13], and the same precursor ions and product ions were used for SRM in this study. Briefly, the ion at *m/z* 125, the major fragment ion for both ketamine and norketamine, was chosen as the product ion in the SRM scan for the two compounds. The selected reaction *m/z* 238 → *m/z* 125 for ketamine was monitored with a collision energy of 25 eV and a dwell time of 0.2 s; the selected reaction *m/z* 224 → *m/z* 125 for norketamine was monitored with a collision energy of 20 eV and the same dwell time of 0.2 s; the selected reaction *m/z* 228 → *m/z* 129 for internal standard d_4-norketamine was monitored with the same conditions as for norketamine. The inter-channel delay time and inter-scan delay time were set at 0.02 s and 0.1 s respectively. The spectra were acquired and processed with the MassLynx™ V4.0 software (Waters, U.S.A).

3. Results and discussion

3.1. Analysis of protein solutions containing salts or detergents

A solution of cytochrome c (5 μM) containing 1% NaCl was analyzed by C18 pipette-tip ESI-MS, in comparison with conventional ESI-MS. As shown in Fig. 2A and B, only the NaCl clusters and

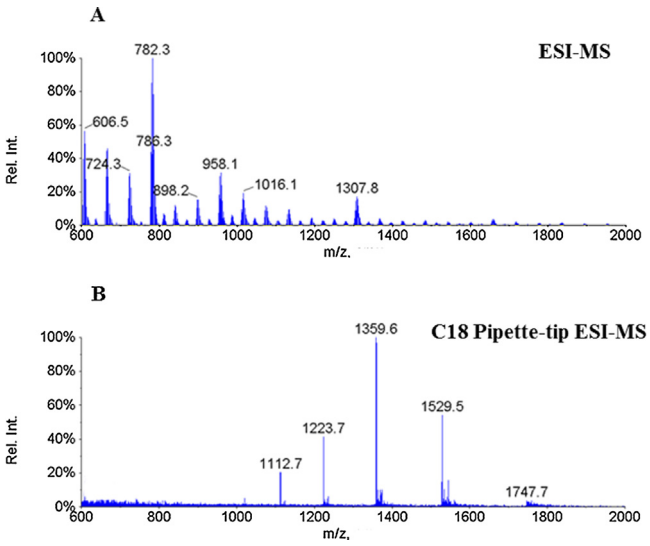

Fig. 2. Spectra obtained by analysis of cytochrome c (5 μM, 1% NaCl) with conventional ESI-MS (**A**) and C18 pipette-tip ESI-MS (**B**).

no signals of the protein could be detected in the mass spectrum when the sample was analyzed by conventional ESI-MS. While with C18 pipette-tip ESI-MS, multiply charged ions (±7 to ±11) of cytochrome c were clearly observed in the spectrum with almost no signal of the salt. Similar results were obtained when another protein solution, 5 μM myoglobin with 1% NaCl, was analyzed. As shown in Fig. 3A and B, no peaks corresponding to myoglobin were observed in the spectrum obtained with conventional ESI-MS; while with C18 pipette-tip ESI-MS, a quality spectrum showing a series of multiply charged ions (±11 to ±21) of myoglobin and the heme (*m/z* 616.2) was obtained. These results demonstrated the capability of C18 pipette-tip ESI-MS for desalting and rapid analysis of protein samples containing salts.

Protein solutions containing detergents were examined as well in this study, and the spectral results are shown in Fig. 4. α-Lactalbumin, a common membrane protein, in a solution containing 0.1% SDS was analyzed using both conventional ESI-

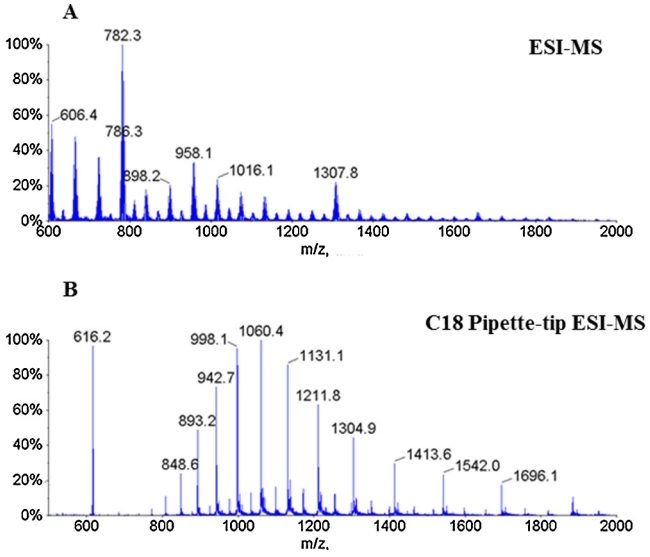

Fig. 3. Spectra obtained by analysis of myoglobin (5 μM, 1% NaCl) with conventional ESI-MS (**A**) and C18 pipette-tip ESI-MS (**B**).

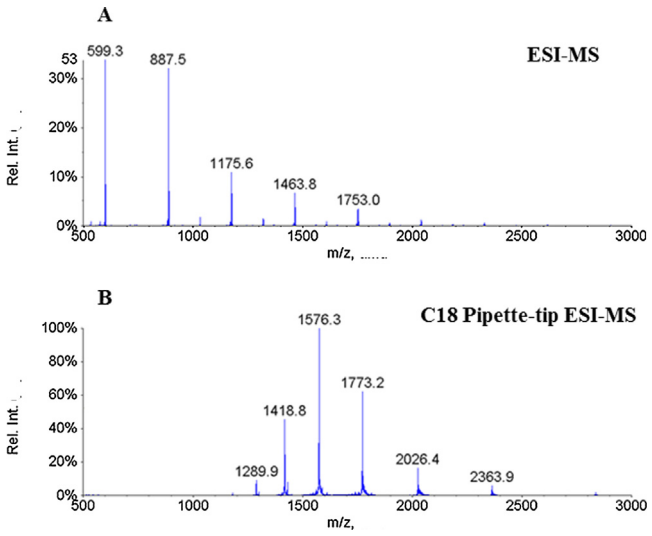

Fig. 4. Spectra obtained by analysis of α-lactalbumin (5 μM, 0.1% SDS) with conventional ESI-MS (**A**) and C18 pipette-tip ESI-MS (**B**).

MS and C18 pipette-tip ESI-MS. Conventional ESI-MS could not detect any protein signals and only the SDS clusters could be obtained. With C18 pipette-tip ESI-MS, the multiply charged ions (±6 to ±11) of α-lactalbumin could be detected obviously.

The above results indicated that relatively hydrophobic proteins could be retained by nonpolar C18 sorbent, while hydrophilic NaCl and SDS had less affinity with the sorbent and could be readily washed out. The results also demonstrated that C18 pipette-tip ESI-MS could be used for rapid and convenient detection of proteins from solutions containing salts and detergents.

3.2. Quantitation of ketamine and norketamine in urine

We first tested the possibility to repeatedly use one C18 pipette tip for the measurements. The reproducibility using three individual C18 pipette tips or the same C18 pipette tip for three repeat measurements was investigated with a urine sample spiked with 50 ng mL^{-1} ketamine, norketamine and the internal standard, and the obtained SRM chromatograms are shown in Fig. 5. For three individual C18 pipette tips, the RSD (relative standard deviation) for the three peak area ratios of ketamine and norketamine to the internal standard was 10.2% and 9.8% respectively, which are acceptable for sample analysis. For the repeat measurements using the same C18 pipette tip, the peak area ratios for ketamine and norketamine were 11.6% and 13.1% respectively, which were in acceptable ranges and indicated no memory effects from the previous measurement. To reduce the experiment costs and make the measurement simpler, one C18 pipette tip was used for the whole measurements. The re-used C18 pipette tip was carefully washed with 200 μL methanol/

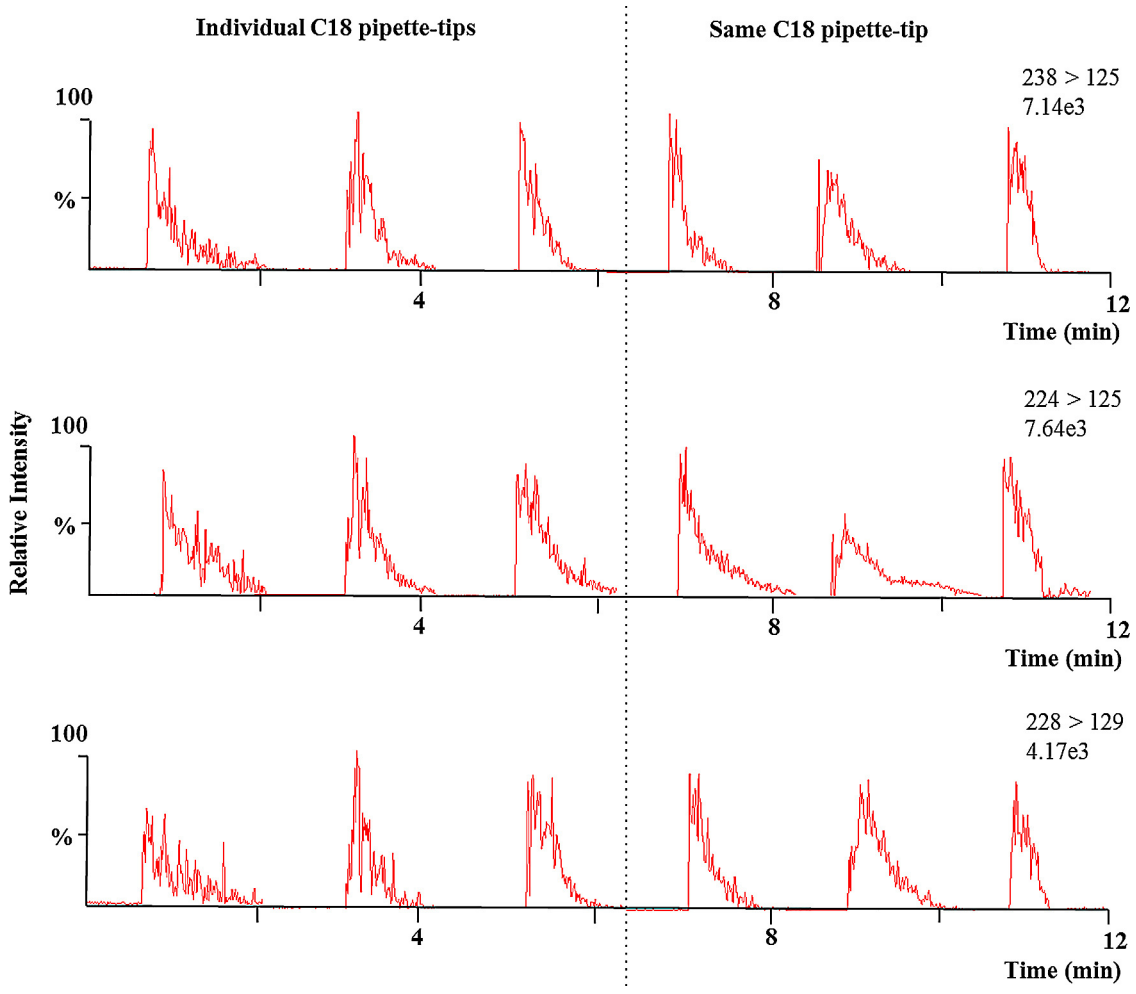

Fig. 5. The SRM chromatograms of three measurements using three individual C18 pipette tips (left) and the same C18 pipette tip (right).

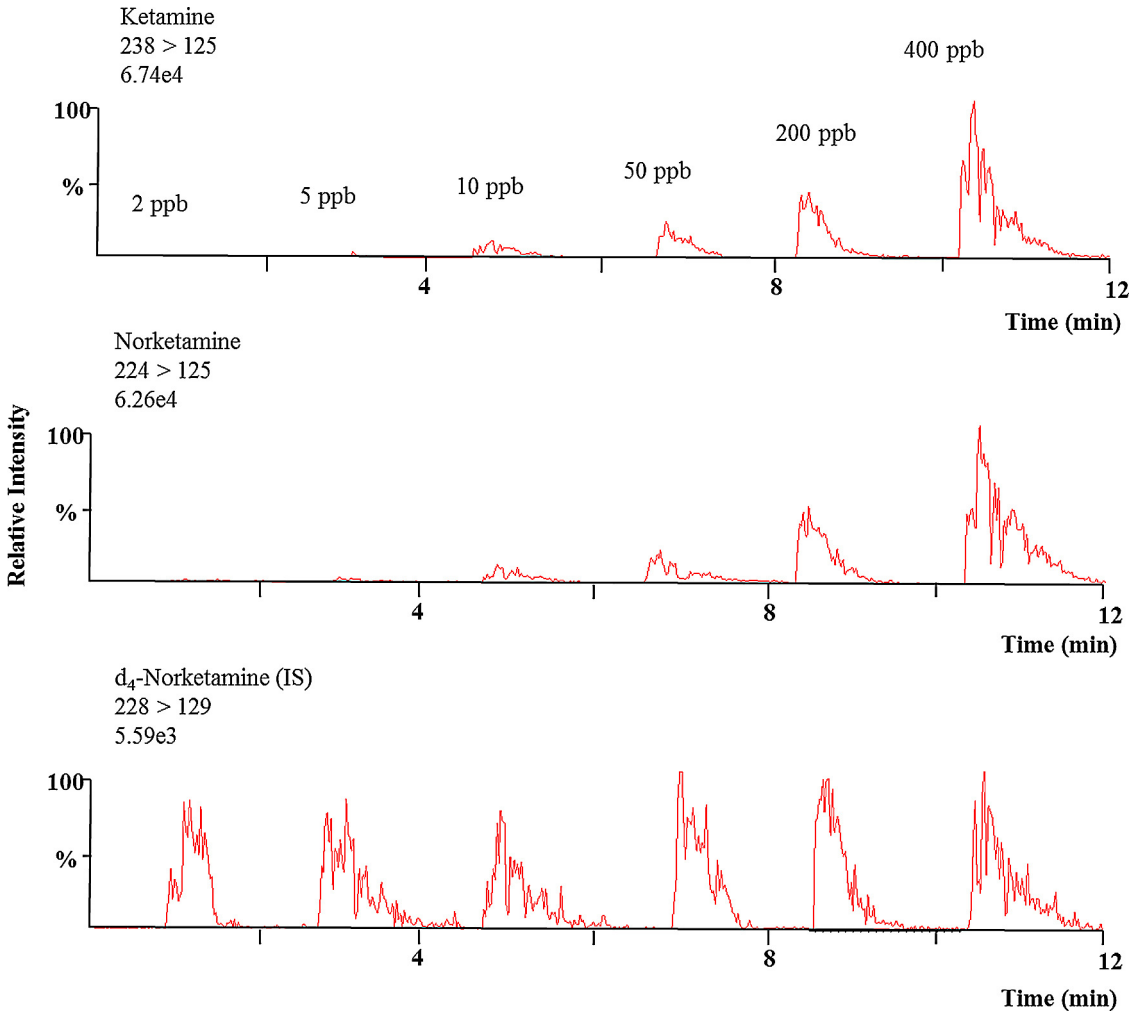

Fig. 6. The SRM chromatograms for simultaneous detection of ketamine (m/z 238 > 125, 2–400 ng mL^{-1}), norketamine (m/z 224 > 125, 2–400 ng mL^{-1}) and the internal standard d_4-norketamine (m/z 228 > 129, 50 ng mL^{-1}).

water/1% formic acid (50/50/1, v/v/v) each time before the measurement.

Quantitation of ketamine and norketamine in urine was simultaneously performed with C18 pipette-tip ESI-MS, using d_4-norketamine as the internal standard and selected reactions m/z 238 → m/z 125, m/z 224 → m/z 125, and m/z 228 → m/z 129 for the three compounds respectively. The C18 pipette tip allowed rapid sample enrichment and clean-up. A lower concentration range of 2–400 ng mL^{-1} was thus investigated with this novel technique. The SRM chromatograms for ketamine (m/z 238 > 125) and norketamine (m/z 224 > 125) in the concentration sequence of 2, 5, 10, 50, 200, 400 ng mL^{-1} and the internal standard d_4-norketamine (m/z 228 > 129) with a fixed concentration of 50 ng mL^{-1} are shown in Fig. 6. A positive correlation between the peak area and analyte concentration was found both in the analysis of ketamine and norketamine. The peak area of the internal standard for different samples did not vary significantly. As shown in Fig. 6, distinct chromatographic peaks with a time window of 40–80 s could be obtained. Since the sample preparation procedure with the C18 pipette tip only needed about 2–3 min for each sample, the total time for analysis of each sample could be less than 5 min.

For construction of calibration curves, each spiked urine sample was measured for three times, and the mean values of the peak

Table 1
Data for quantitation of ketamine and norketamine.

Concentration (ng mL^{-1})	Ketamine		Norketamine	
	Peak area ratio (analyte/IS)	RSD (n = 3, %)	Peak area ratio (analyte/IS)	RSD (n = 3, %)
2	0.1066	3.05	0.0978	11.13
5	0.1755	3.02	0.1804	7.60
10	0.3070	3.02	0.4966	4.47
50	1.6935	5.04	2.1889	8.50
100	3.1496	5.82	4.0405	6.15
400	12.8589	5.43	16.7950	8.03

Table 2
Precision and accuracy for quantitation of ketamine and norketamine in urine with C18 pipette-tip ESI-MS.

Spiked concentration ($ng\,mL^{-1}$)	Ketamine			Norketamine		
	Measured concentration ($ng\,mL^{-1}$)	Precision ($n=6$)	Accuracy ($n=6$)	Measured concentration ($ng\,mL^{-1}$)	Precision ($n=6$)	Accuracy ($n=6$)
20	18.9	13%	94%	19.6	5%	98%
200	179.2	5%	90%	177.1	7%	89%

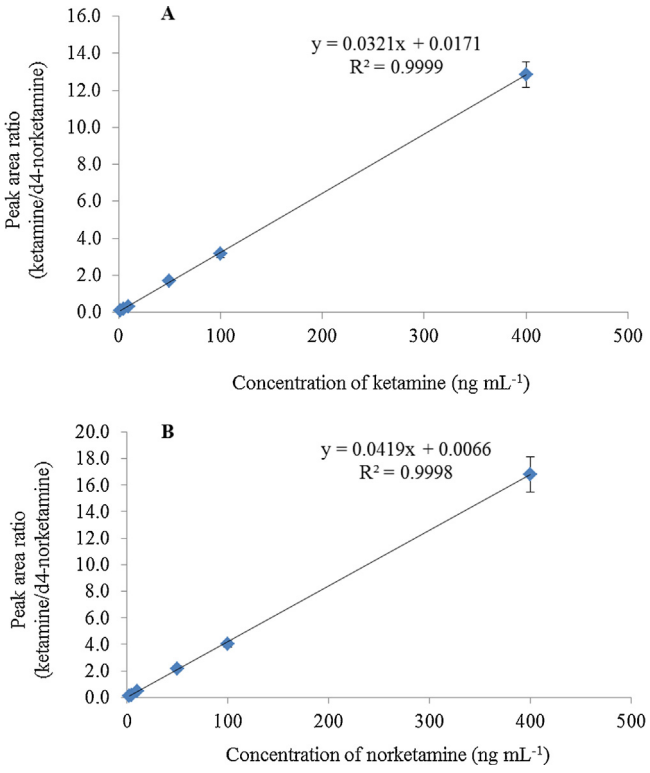

Fig. 7. Calibration curves for ketamine (**A**) and norketamine (**B**).

area ratios between analyte and the internal standard and their RSDs are shown in Table 1. The calibration curves for ketamine and norketamine, as shown in Fig. 7, were obtained by plotting peak area ratio against the analyte concentration. For both ketamine and norketamine, the calibration curves displayed excellent linear relationship over the examined concentration range of 2–400 ng mL^{-1}. The coefficient R^2 was very close to 1 for both ketamine ($R^2 = 0.9999$) and norketamine ($R^2 = 0.9998$). This linear range covered the concentration ranges of ketamine and norketamine in common drug abuser's urine [29], indicating the applicability of the present technique in real applications. This achieved linear range was comparable to the conventional HPLC–MS and GC–MS in previous reports [13,27,28,31–33]. The RSDs for analysis of each sample are listed in Table 1, which were acceptable for measurements.

The precision and accuracy for quantitation of ketamine and norketamine in urine with C18 pipette-tip ESI-MS were investigated as well (Table 2). A low concentration at 20 ng mL^{-1} and a high concentration at 200 ng mL^{-1} were measured. For ketamine, the precisions were determined to be 13% and 5%, and the accuracies were determined to be 94% and 90%, at the two concentrations respectively. For norketamine, the precisions were 5% and 7%, and accuracies were 98% and 89%, at the two concentrations respectively. These results were comparable to those obtained with conventional methods [13,27,28,31–33].

The limit-of-detection (LOD) and limit-of-quantitation (LOQ) of this method were determined by comparing the peak height of the analyte with the average height of the noises after the analyte peak. The LOD and LOQ were defined as the concentration of the analyte when the signal of the analyte is three and ten times of the signal of the noises respectively. The LOD and LOQ for the present technique were determined to be 0.3 ng mL^{-1} and 0.5 ng mL^{-1}, respectively, for ketamine and 0.8 ng mL^{-1} and 1.0 ng mL^{-1}, respectively, for norketamine, The LOD and LOQ achieved here were comparable to those by the conventional methods [34–36] and good enough for real applications, although only simple sample cleanup followed by direct MS detection were involved.

4. Conclusions

By combining C18 pipette tip, a device for rapid purification of samples, with syringe and syringe pump, a C18 pipette-tip ESI-MS technique was developed. In this technique, analyte in raw solution samples was concentrated and purified by C18 pipette tip rapidly and directly eluted to generate electrospray ionization for mass spectrometric analysis. Continuous and controllable supply of solvents by the syringe pump allowed observation of stable and reproducible signals. Using this novel technique, rapid detection of proteins from solutions containing salts such as sodium chloride or detergents such as SDS, and quantitation of ketamine and norketamine in human urine with desired analytical performance, were successfully achieved. The linear range, precision, accuracy, LOD and LOQ for quantitation of both ketamine and norketamine with this method were well acceptable for analysis of real samples. This method is very simple, easy to operate, cost-effective (the C18 pipette tip could be reused), and thus very useful for rapid analysis of raw solution samples. The C18 sorbent can also be replaced with other chromatographic materials and be used for other analytical purposes.

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