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Original Article

Rapid detection and quantitation of drugs-of-abuse by wooden-tip electrospray ionization mass spectrometry

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ABSTRACT

Determination of drugs-of-abuse in body fluids of drug abusers is important for the law enforcement as well as the treatment and rehabilitation. In this study, wooden-tip electrospray ionization mass spectrometry (WT-ESI-MS), a simple and cost-effective technique, was developed for rapid detection and quantitation of common drugs-of-abuse, including methamphetamine, methylenedioxymethamphetamine (MDMA), cocaine, heroin and tetrahydrocannabinol (THC), in urine and oral fluid, following our previous successful demonstration for rapid and sensitive detection of ketamine and nor-ketamine in urine and oral fluid by this technique. It was found that the limit-of-detection for methamphetamine could fully fulfill the cut-off value requirements of the international standards, and those of MDMA and cocaine could fulfill some of the requirements. The linear range, accuracy and precision for quantitation of the drugs were generally satisfactory, except for THC for which the analytical performance still needs to be improved. Analysis of one sample could typically be completed within minutes. These results indicated that WT-ESI-MS could be used for rapid screening of drugs-of-abuse in urine, oral fluid as well as other body fluids.

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1. Introduction

Determination of drugs-of-abuse and their metabolites in body fluid is an essential task for drug abuse control. Drug analysis of a large number of body fluid samples is required for law enforcement and healthcare purposes each year. To deal with this, typically the body fluid samples are firstly analyzed by fast screening methods, such as antibody-based screening devices and immunoassay methods [1–5]. However, these methods have a number of problems, including cross-reactivity [2,3,5,6] and generation of false positive and false negative results [3,5–10]. Therefore, confirmatory analysis by using gas chromatography-mass spectrometry (GC–MS) and liquid chromatography-mass spectrometry (LC-MS) was normally performed afterwards [1,3,5,11–15]. These routine methods typically require extensive sample pre-treatment and chromatographic separation, which can be time-consuming and labor-intensive.

Recently, various efforts have been made to minimize the sample pre-treatment and eliminate the chromatographic separation of drugs analysis. For example, electrospray-assisted laser desorption/ionization mass spectrometry (ELDI-MS) [16], desorption electrospray ionization mass spectrometry (DESI-MS) [17,18], desorption atmospheric pressure photoionization-mass spectrometry (DAPPI-MS) [19], paper spray ionization mass spectrometry (PS-MS) [20,21], probe electrospray ionization mass spectrometry (PESI-MS) [22], pipette-tip electrospray ionization mass spectrometry [23] and touch spray mass spectrometry (TS-MS) [24] were developed for rapid detection of drugs-of-abuse.

Wooden-tip electrospray ionization mass spectrometry (WT-ESI-MS) is a technique that makes use of economical and commonly available wooden toothpicks for sample loading

Table 1 – MRM conditions and cone voltages for WT-ESI-MS analysis of various drugs, metabolites, and deuterium-labeled internal standards.

Analyte	MRM channel	Collision cell energy (V)	Cone voltage (V)
MA	150 → 91	15	30
D-MA	155 → 121	10	30
MDMA	194 → 163	8	30
D-MDMA	199 → 165	10	30
COC	304 → 182	15	30
D-COC	307 → 185	15	30
BZE	290 → 168	18	30
D-BZE	293 → 171	18	30
THC	315 → 193	30	30
D-THC	318 → 196	25	30
THC-COOH	343 → 299	25	30
D-THC-COOH	352 → 308	25	30
HER	370 → 268	28	45
6-AM	328 → 165	35	40
D-6-AM	331 → 165	32	35
MOR	286 → 165	38	40

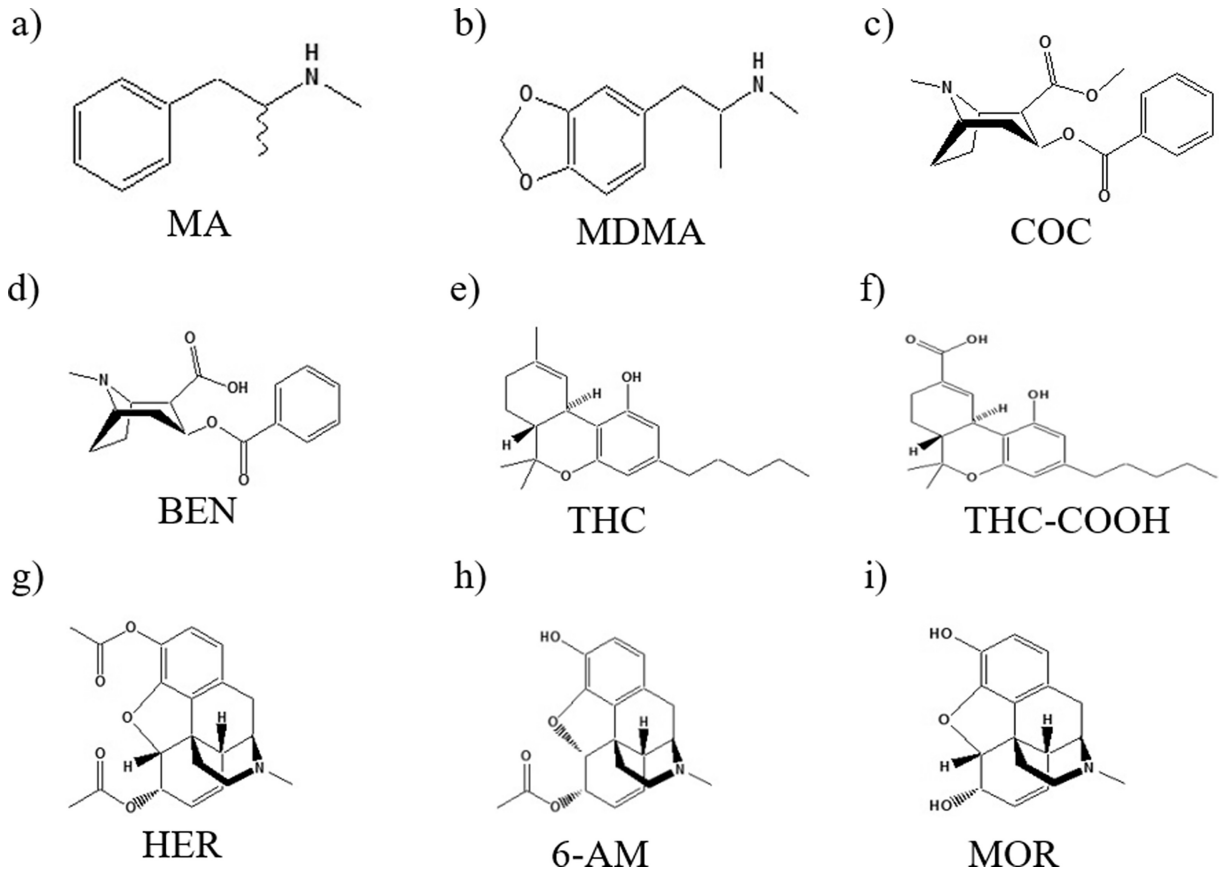

Fig. 1 – Molecular structures of (a) methamphetamine, (b) MDMA, (c) cocaine, (d) benzoylecgonine, (e) THC, (f) THC-COOH, (g) heroin, (h) 6-acetylmorphine and (i) morphine.

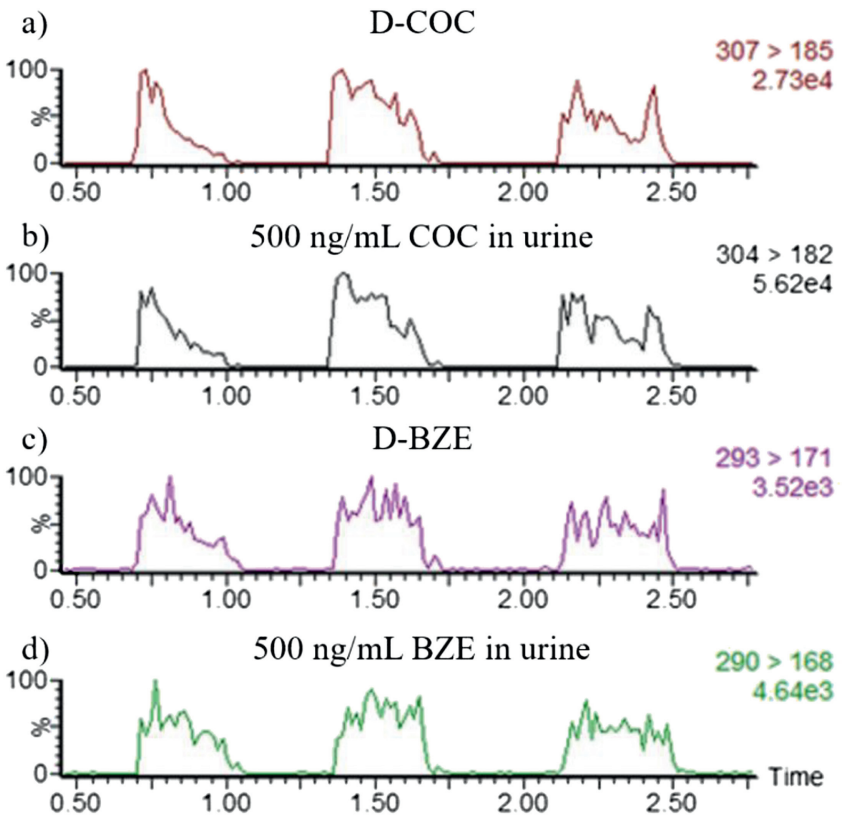

Fig. 2 – MRM results for detection of (a) cocaine-D_3, (b) 500 ng/mL cocaine, (c) benzoylecgonine-D_3 and (d) 500 ng/mL benzoylecgonine in urine using WT-ESI-MS.

and ionization [25]. Typical commercially available wooden tips are directly compatible with the nano-ESI source and thus no hardware modification is needed for the technique, making the technique easily adopted by related researchers. WT-ESI-MS was found to allow analysis of raw biological samples with only simple sample preparation and no column separation. Therefore, analysis of one raw sample could be completed within minutes, and the technique has been proven for various applications [26–32]. We have demonstrated that WT-ESI-MS could be applied for rapid detection and quantitation of ketamine and nor-ketamine in urine and oral fluid [33]. In this study, we further extended the application of WT-ESI-MS for rapid detection and quantitation of other common drugs-of-abuse, including methamphetamine (MA), cocaine (COC), methylenedioxymethamphetamine (MDMA), tetrahydrocannabinol (THC), heroin (HER), and their metabolites, in urine and oral fluid.

2. Experimental

2.1. *Materials and chemicals*

The wooden toothpicks used in this study were BEST-Buy brand purchased from the supermarket in Hong Kong. Methamphetamine (MA), methamphetamine-D5 (D-MA), MDMA, MDMA-D5 (D-MDMA), cocaine (COC), cocaine-D3 (D-COC), benzoylecgonine (BZE), benzoylecgonine-D3 (D-BZE), delta-9-THC (THC), delta-9-THC-D3 (D-THC), 11-nor-9-carboxy-delta-9-THC (THC-COOH), 11-nor-9-carboxy-delta-9-THC-D9 (D-THC-COOH), heroin (HER), 6-acetylmorphine (6-AM) and 6-acetylmorphine-D3 (D-6-AM) standard solutions were purchased from Cerilliant (Round Rock, TX) and morphine (MOR) sulphate salt solution was purchased from Fluka (St. Louis, TX). The molecular structures of these analytes are shown in Fig. 1. HPLC grade methanol was purchased from Tedia (Fairfield, CT) and formic acid was purchased from Sigma (St. Louis, TX).

2.2. *Sample preparation*

The standard solutions of the targeted drugs, metabolites and internal standard used for the construction of calibration curve were prepared by serial dilution of stock standard solutions of each drug with methanol containing 0.1% formic acid. At least five concentrations were prepared for constructing the calibration curves. Another set of standard solutions with low, middle and high concentrations was prepared for the method validation. The standard solutions of drugs-of-abuse and the related metabolites were spiked into blank urine and oral fluid to prepare the spiked samples. Finally, the spiked samples, internal standard solutions, and methanol with 0.1% formic acid were mixed in the ratio of 1:1:1 (v/v/v), and the prepared samples were ready for WT-ESI-MS analysis. All the spiked sample solutions were freshly prepared before the analysis.

2.3. *Instrumentation and WT-ESI-MS setup*

The WT-ESI-MS analysis was performed on a Quattro Ultima triple quadrupole mass spectrometer (Waters, Milford, MA) with a nano-ESI source. A sharpened wooden tip with a length of 1.5–1.7 cm was mounted onto the capillary holder and a high voltage (3.5 kV) was applied to the tip directly from the nano-ESI source. An aliquot of 2 μL sample solution was then applied to the tip by pipetting for WT-ESI-MS analysis. Typically, each sample was analyzed three times by using the same wooden tip. The mass spectrometer was operated in positive ion mode, except negative ion mode for the analysis of THC-COOH. The cone gas flow and source temperature were 100 L/hr and 150 °C, respectively. The drugs-of-abuse and their metabolite were detected under multiple reaction monitoring (MRM) mode, and the ion channels, collision energy and cone voltage used are listed in Table 1.

2.4. *Method validation of WT-ESI-MS*

2.4.1. *Calibration curves*

The calibration curves were constructed by averaging three sets of the experimental data with each set of data contained at least five calibration points. The resultant MRM chromatograms were processed using the Mass Lynx 4.1 program (Milford, MA). The MRM chromatograms were smoothed, and the averages of peak height ratios of the analytes and the internal standards were used for constructing the calibration curves.

2.4.2. *Accuracy and precision*

The accuracy and precision of the WT-ESI-MS method were determined by measuring at least three sets of the spiked urine and oral fluid samples at low, medium, and high concentrations, respectively. The sample at each concentration was analyzed at least five times using individual wooden tips. The accuracy was defined as the closeness of the measured concentration (C_m) and the actual concentration of the analyte in the sample (C_a) [34] and calculated as following: (C_m/C_a) x 100%, and the precision was represented by relative standard deviation (RSD).

2.4.3. *Limits-of-detection and limits-of-quantitation*

Limits-of-detection (LODs) and limits-of-quantitation (LOQs) were determined experimentally by comparing the signals of spiked samples at different concentrations and signals obtained from blank samples. The blank samples for the

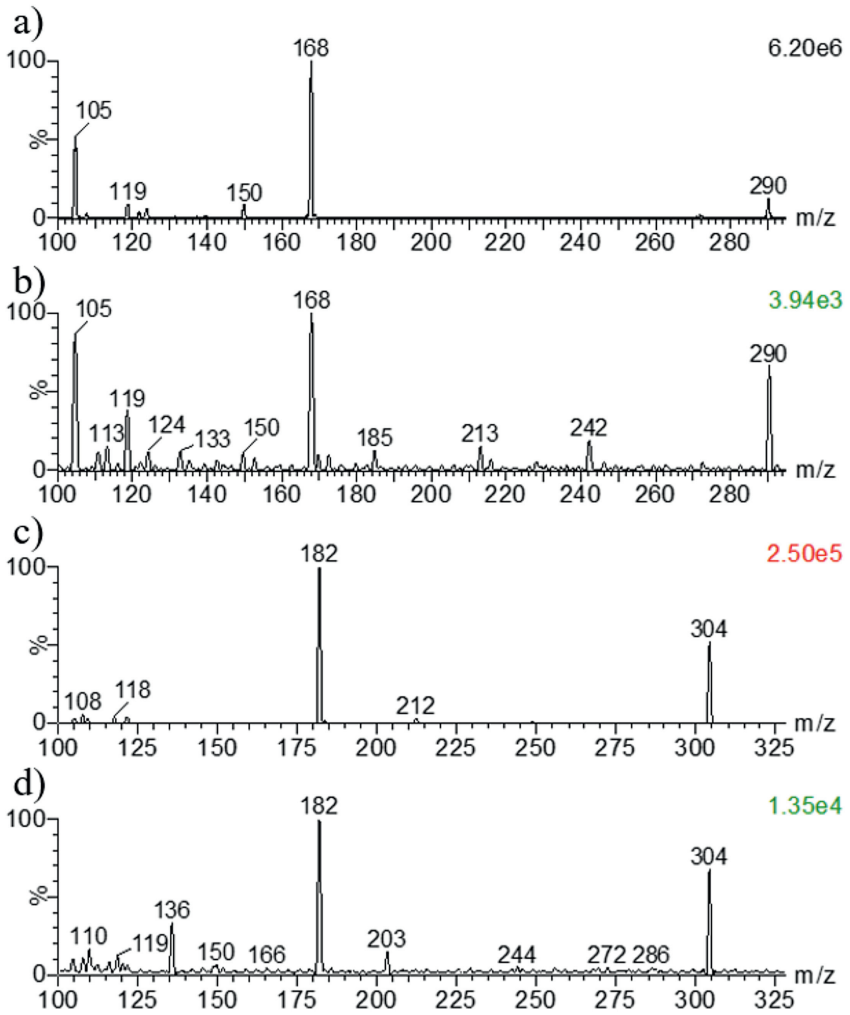

Fig. 3 – MS/MS spectra of 1000 ng/mL standard solution of benzoylecgonine (a) and cocaine (c), 250 ng/mL benzoylecgonine in urine (b) and 50 ng/mL cocaine in urine (d).

background signal measurements were prepared by spiking only the internal standards into blank urine and blank oral fluid. The LODs and LOQs were defined as the concentrations of the analytes that could achieve signal-to-noise ratios (S/N) at the factors of three and ten, respectively. The S/N were determined by comparing the signal intensities (in term of peak heights) of the analytes (I_a) and internal standards (I_{is}) between the spiked samples and the blank samples, and calculated as following: $(I_a/I_{is})_{spiked}/(I_a/I_{is})_{blank}$. At least nine blank measurements were used for the determination of LODs and LOQs.

3. Results and discussions

3.1. Detection of drugs-of-abuse

Typical MRM results for the detection of cocaine and its metabolite benzoylecgonine in urine are shown in Fig. 2. The signals of the analytes were generated directly after applying the sample solution to the wooden tip connected with a high voltage. Deuterium-labeled internal standards of the corresponding analytes were added to compensate the signal fluctuations caused by different wooden tips and other variations. The targeted drugs, metabolites and internal standards were detected simultaneously under the MRM detection without chromatographic separation. Each signal could typically maintain 10–30 s, and each sample was applied onto the same wooden tip three times. Signals were considered as positive if the S/N values were larger than 3 as compared with the blank (see the later part for further discussion). The identities of the analytes could be further confirmed by tandem mass spectrometry (MS/MS) analysis. Examples of MS/MS analysis are shown in Fig. 3. The MS/MS spectrum of standard solution of benzoylecgonine (Fig. 3a) is similar to that of benzoylecgonine in urine (Fig. 3b). Both spectra showed major product ions of benzoylecgonine including *m/z* 168, *m/z* 105 and *m/z* 150. Similarly, the major product ion of cocaine, i.e., *m/z* 182, could be observed in both the MS/MS of cocaine standard (Fig. 3c) and cocaine in the urine sample (Fig. 3d).

3.2. Reproducibility of the detection

The reproducibility of the WT-ESI-MS method was examined by measuring the same sample solution repeatedly using individual wooden tips at the same and different day. Typical results are shown in Fig. 4, using methamphetamine as an example. The absolute intensities of the measurements using different wooden tips varied but were within an acceptable range. The precision of all the measurements (i.e., data from four individual wooden tips, n = 12) was 15.0%. The precisions of the measurements were improved after the addition of the internal standards. The precisions of the measurements, in term of relative peak height (i.e., the peak heights of the

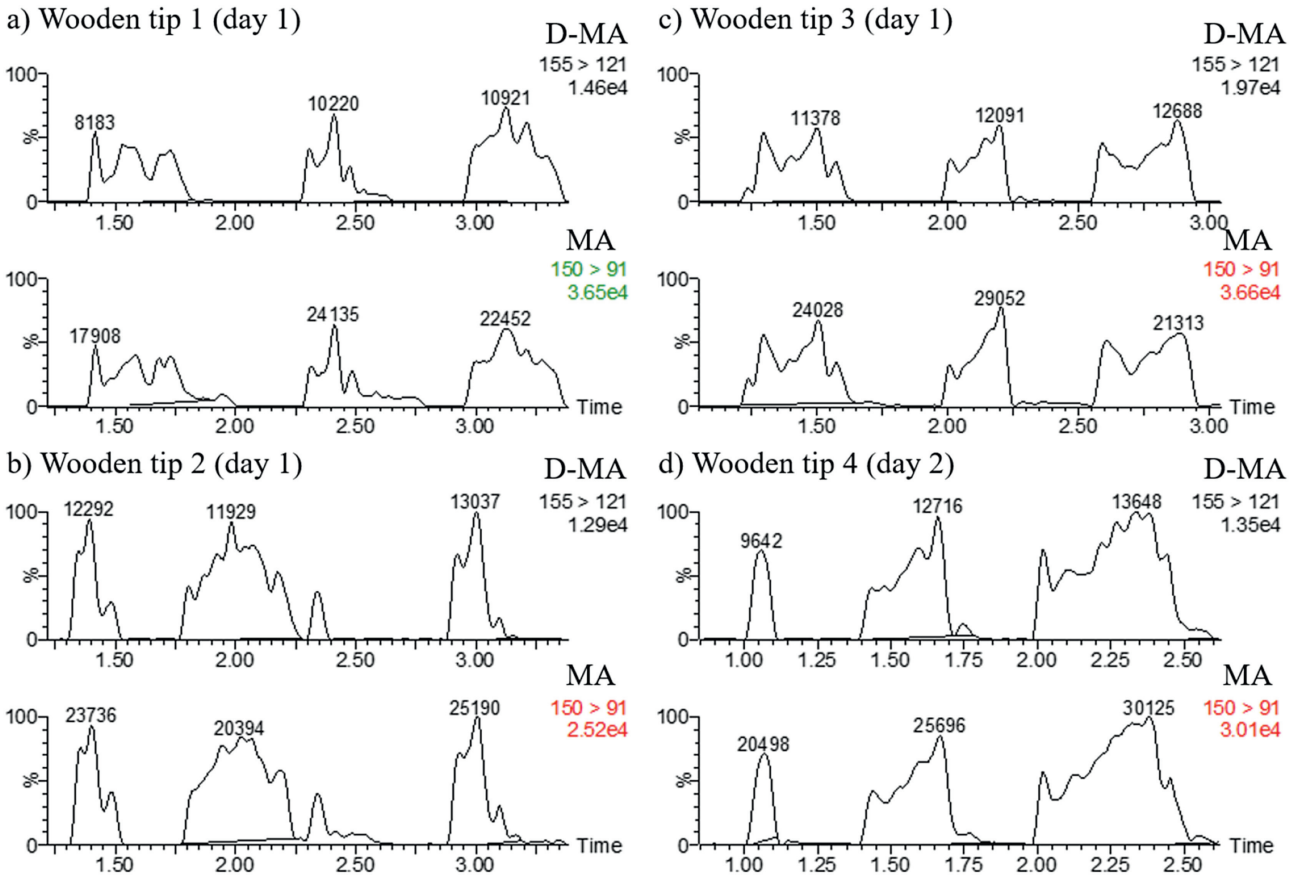

Fig. 4 – MRM results for detection of 500 ng/mL methamphetamine in urine using (a–c) three individual wooden tips within the same day and (d) individual wooden tip on another day.

analytes divided by the peak heights of the internal standards), was 10.9%. Therefore, addition of the internal standards is recommended for the WT-ESI-MS analysis.

3.3. *Quantitation of targeted analytes*

3.3.1. *Calibration curves*

The calibration curves used for quantitative analysis were obtained by correlating the signals of at least five spiked samples with different concentrations. The constructed calibration plots of different analytes in urine and oral fluid are shown in Figs. 5 and 6, respectively. Deuterated heroin and morphine were not available during the study period, and D-6-AM was used as the internal standards of heroin and morphine instead. The linear range of most of the analytes could cover three orders of magnitude (typically 50–5000 ng/mL) except for heroin and its metabolites (typically 250–10,000 ng/mL), and benzoylecgonine in urine (125–5000 ng/mL). The R^2 values of all the calibration curves were greater than 0.99, indicating the good linearity of the calibration plots. The precisions of the

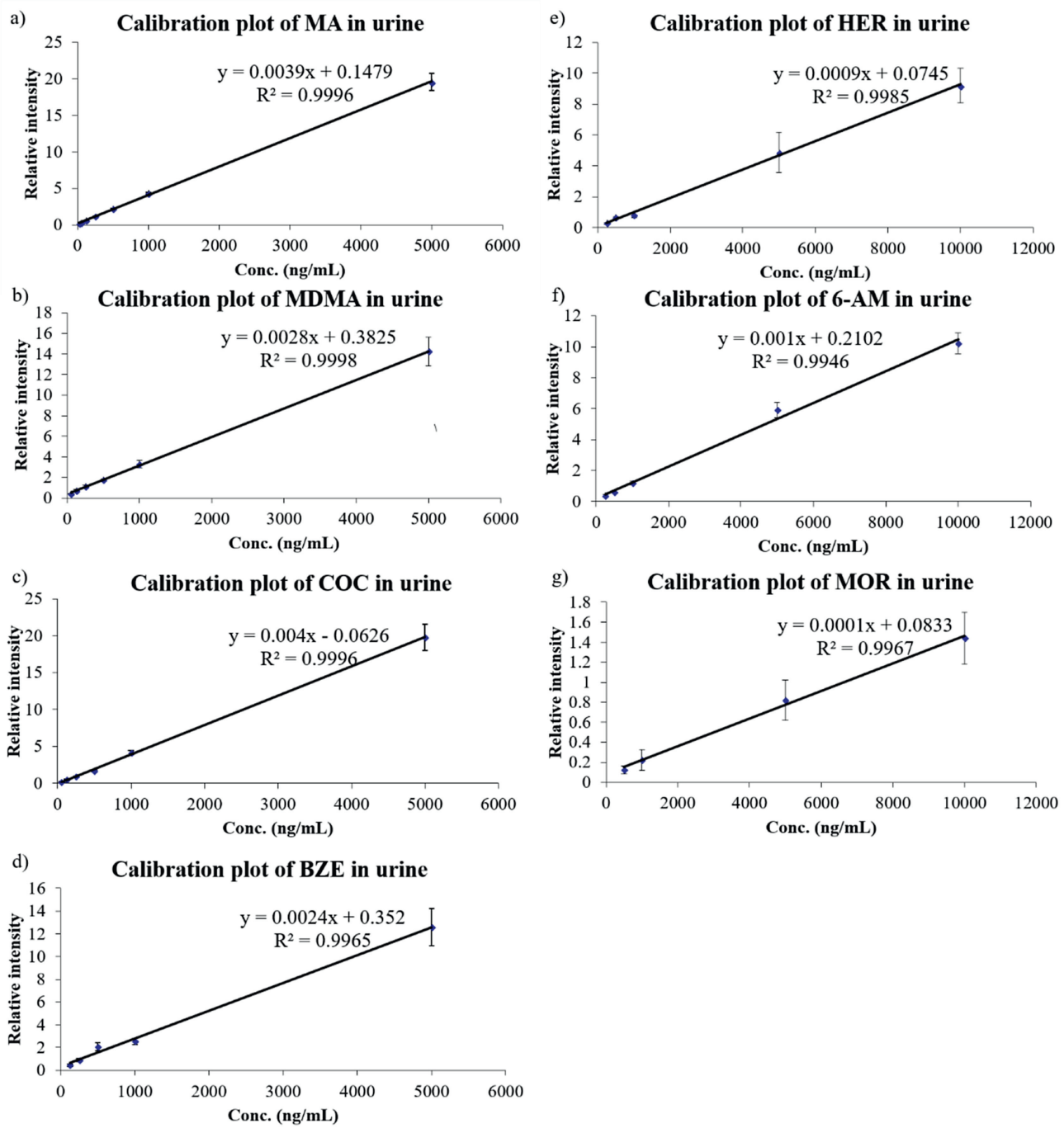

Fig. 5 – Calibration plots for quantitation of (a) methamphetamine, (b) MDMA, (c) cocaine, (d) benzoylecgonine, (e) heroin, (f) 6-acetylmorphine and (g) morphine in urine.

calibration points were better than 20%, except for morphine with the average RSD of 25.9% and 52.6% in urine and oral fluid, respectively. This was believed to be due to the fact that the signals of morphine were much lower and unstable when compared with those of other analytes. It was also found that the ionization of THC and THC-COOH was poor with the present method, and their signals could only be produced at significantly high concentrations. Therefore, no corresponding calibration curves could be constructed for THC and THC-COOH.

3.3.2. *Accuracy and precision*

The accuracy and precision of the WT-ESI-MS method for quantitation of targeted analytes were evaluated using the spiked samples at low, middle and high concentrations in urine and oral fluid, and the results are summarized in Table 2. The accuracies of the WT-ESI-MS method for analysis of all analytes were in the range of 83.8–117.1%, except 78.2–113.7% and 75.2–109.9% for heroin and morphine, respectively. The differences between the spiked values and measured values were generally within ±20%. The precisions of

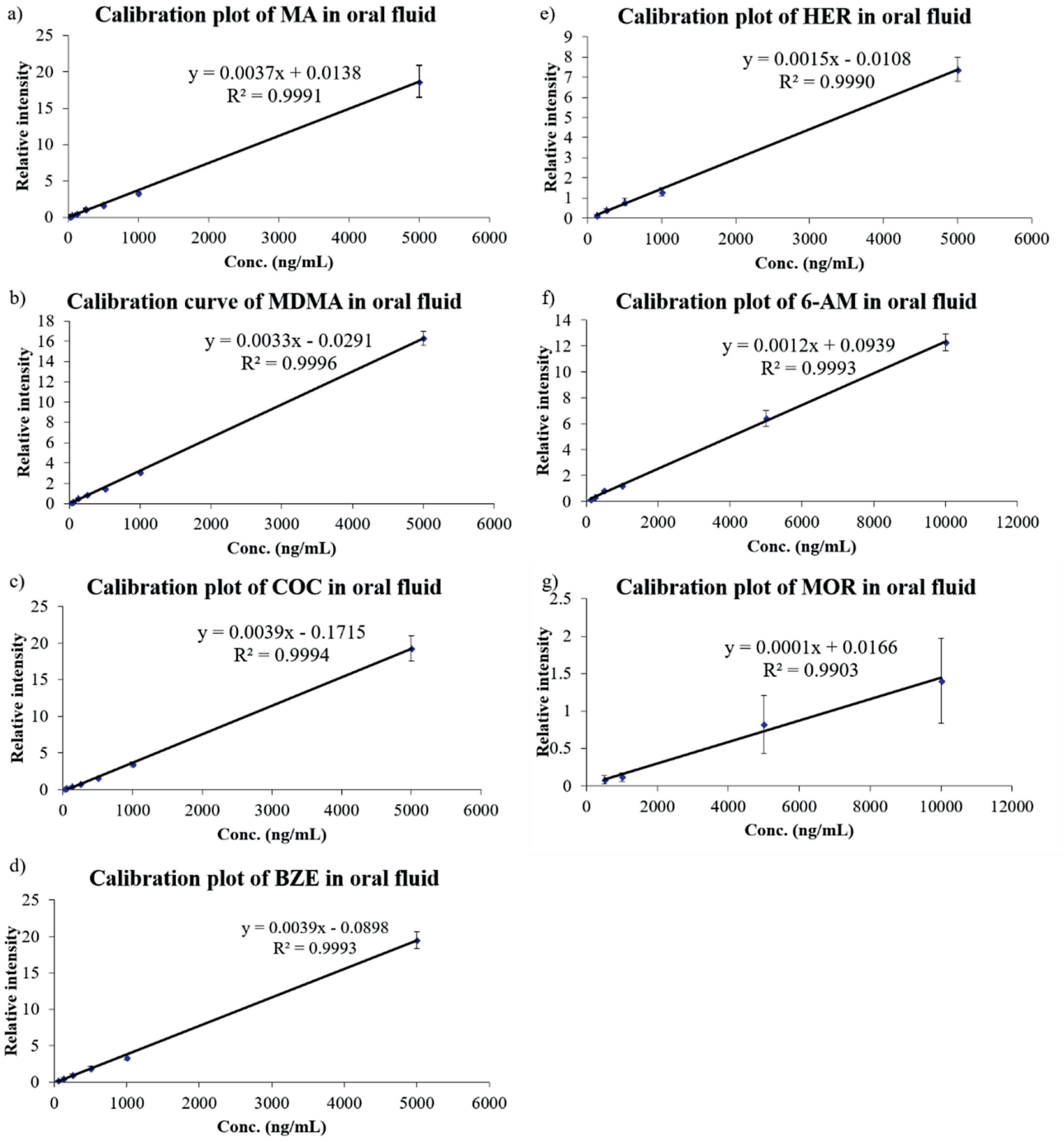

Fig. 6 – Calibration plots for quantitation of (a) methamphetamine, (b) MDMA, (c) cocaine, (d) benzoylecgonine, (e) heroin, (f) 6-acetylmorphine and (g) morphine in oral fluid.

methamphetamine, MDMA, cocaine, and benzoylecgonine were generally within 15%, except 17.0% for methamphetamine in urine. The precisions of heroin and its metabolites were generally slightly greater than the values of other analytes but the RSD was still within 20%. The results obtained from morphine were generally the worst, with the accuracy of only 75.2% and the values of precision higher than 20% even at high concentrations. The data of accuracy and precision of THC and THC-COOH were not available because of the poor sensitivity of the detection. In general, the accuracy and precision for quantitation of most of the targeted analytes, except morphine, THC, and THC-COOH, were satisfactory using the WT-ESI-MS method.

3.3.3. *LODs and LOQs*

An example for the determination of LOD and LOQ of methamphetamine in urine is shown in Fig. 7. The LOD and LOQ of methamphetamine in urine were determined as 25 ng/mL and 50 ng/mL, which could give S/N with the factors of three and ten respectively when compared with the blank samples.

The LODs and LOQs of the targeted analytes determined were compared with the cut-off values of the international authorities, including Substance Abuse and Mental Health Services (SAMHSA) in USA [35], European Workplace Drug Testing Society (EWDTS) [36,37] and Driving under the Influence of Drugs, Alcohol and Medicines (DRUID) project in European Union [38]. The results are summarized in Table 3. The recommended cut-off values for detection of drugs-of-abuse in oral fluid are generally lower than those in urine. There are no recommended cut-off values for ketamine (KET) and nor-ketamine (Nor-K). However, as discussed in the previous study, their LODs were generally good enough for the analysis [33]. The LODs of methamphetamine were within the recommended cut-off values of all the three guidelines. The LODs of MDMA could generally fulfill the requirements but the LOD of oral fluid was slightly higher than the cut-off values of SAMHSA and EWDTS guidelines. For the detection of cocaine, the LOD for analysis of the oral fluid samples could fulfill the requirement of DRUID but was slightly higher than the cut-off values of SAMHSA and EWDTS. The sensitivity of the present method was also not enough for the detection of benzoylecgonine, which is a metabolite of cocaine and is considered as the identifier of cocaine in the SAMHSA and EWDTS guidelines. The detection of heroin related compounds and THC related compounds also needed to be further improved. Especially, very poor signals were obtained for analysis of THC and THC-COOH. Enhanced detection of benzoylecgonine, heroin and related compounds, and THC and THC-COOH may be achieved by using surface-modified wooden tips [39].

Overall, cocaine which is a tertiary amine could give very strong signals and thus its LOD was the lowest. The secondary amines, such as methamphetamine, could also give strong signals and thus low LODs. It is interesting to note that the LOD of cocaine was more than 10 times lower than its metabolite benzoylecgonine, in which an ester group in cocaine was converted to carboxyl group (Fig. 1c and d). The similar situation also occurred for the detection of heroin and its metabolites (Fig. 1g–i). The LOD became higher (i.e., MOR > 6-MAM > HER) when more ester groups were converted to hydroxyl groups. The decreased sensitivity of detection might be due to multiple reasons. First, the analytes were more favorable to retain onto the surfaces of wooden tips with

Table 2 – Accuracy and precision for analysis of various drugs in urine (U) and oral fluid (OF).

Compound	Spiked quantity (ng/mL)	Determined quantity± SD (ng/mL) (n = 5)		Accuracy (%)		RSD (%)	
		U	OF	U	OF	U	OF
MA	100	105 ± 18	114 ± 7	105.7	114.3	17.0	6.3
	500	498 ± 16	508 ± 64	99.5	101.5	3.2	12.6
	1250	1105 ± 60	1216 ± 61	88.4	97.2	5.5	5.0
	2500	2536 ± 151	2518 ± 169	101.4	100.7	6.0	6.7
MDMA	100	112 ± 8	117 ± 5	112.1	117.1	6.8	4.6
	500	520 ± 56	474 ± 28	104.0	94.8	10.7	6.0
	1250	1186 ± 106	1219 ± 82	94.9	97.5	9.0	6.8
	2500	2492 ± 216	2601 ± 392	99.7	104	8.7	15.1
COC	100	103 ± 11	114 ± 11	102.7	114.4	10.9	9.2
	500	510 ± 46	489 ± 58	102.1	97.8	9.0	11.8
	1250	1366 ± 79	1296 ± 166	109.3	103.7	5.8	12.8
	2500	2517 ± 116	2561 ± 319	100.7	102.4	4.6	12.5
BZE	500	432 ± 37	461 ± 61	86.3	92.1	8.6	13.2
	1250	1047 ± 83	1331 ± 137	83.8	106.4	7.9	10.3
	2500	2314 ± 223	2657 ± 209	92.6	106.3	9.6	7.9
HER	500	569 ± 71	515 ± 47	113.7	103.1	12.4	9.2
	1250	1349 ± 247	977 ± 75	107.9	78.2	18.3	7.7
	2500	2585 ± 412	2346 ± 174	103.0	93.8	15.9	7.4
6-AM	500	441 ± 84	467 ± 30	88.1	93.3	19.0	6.4
	1250	1220 ± 62	1024 ± 48	97.6	81.9	5.1	4.7
	2500	2678 ± 267	2822 ± 159	107.1	112.9	10.0	5.6
MOR	500	NA	549 ± 80	NA	109.9	NA	14.6
	1250	1343 ± 342	940 ± 60	107.4	75.2	25.5	6.3
	2500	2456 ± 399	1880 ± 175	98.2	75.2	16.3	9.3

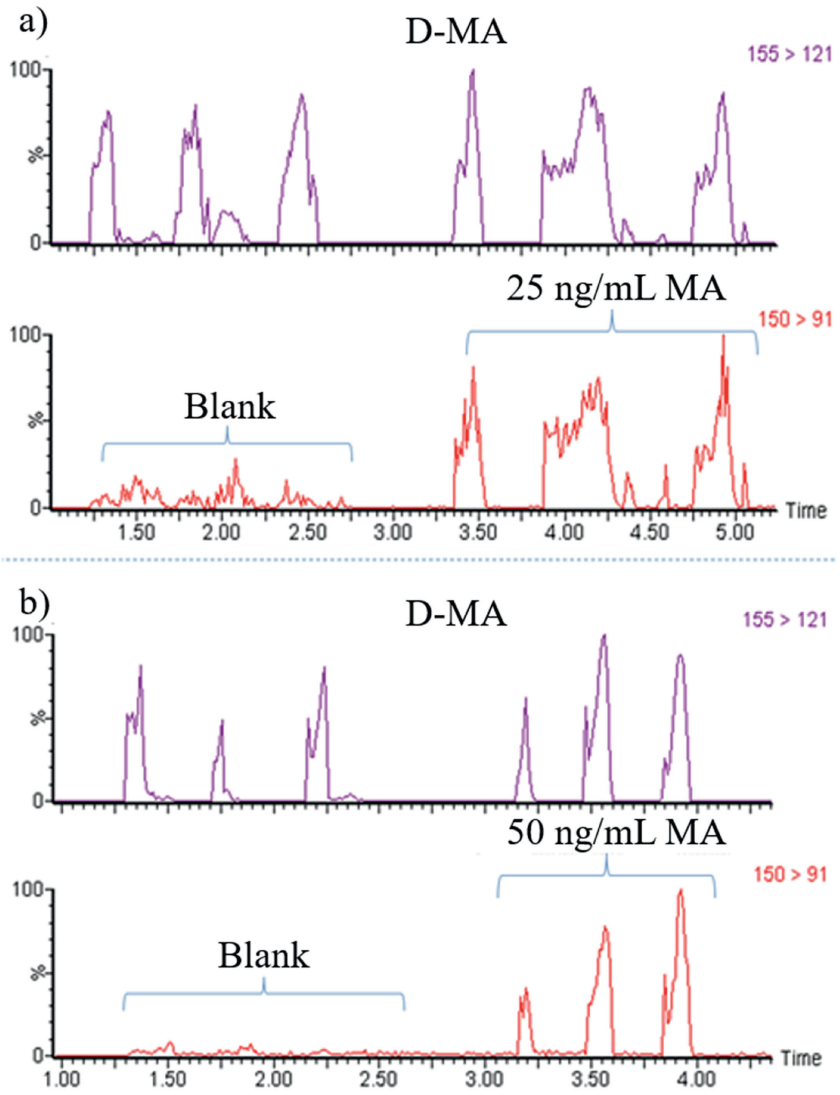

Fig. 7 – Determination of (a) LOD and (b) LOQ of methamphetamine in urine.

Table 3 – LODs, LOQs and recommended cut-off values of common 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)
	U	OF	U	OF	U	OF	U	OF	OF
KET[a]	20	20	50	50	NA	NA	NA	NA	NA
Nor-K[a]	20	20	50	50	NA	NA	NA	NA	NA
MA	25	12.5	50	50	250	15	200	15	410
MDMA	50	50	250	125	250	15	200	15	270
COC	12.5	12.5	50	50	NA	8	NA	8.	170
BZE	250	100	500	250	100	8	100	8	95
THC	40,000	40,000	NA	NA	NA	2	NA	2	27
THC-COOH	NA	NA	NA	NA	15	NA	15	NA	NA
HER	250	125	500	250	NA	NA	NA	NA	NA
6-AM	500	125	1000	250	10	2	10	2	16
MOR	1000	500	10,000	10,000	2000	15	300	15	95

[a] Results adopted from the previous study [33].

the increase of carboxyl group and hydroxyl group, which might have more interactions with the hydroxyl group on the wooden-tip surface. Second, as no chromatographic separation was performed with WT-ESI-MS, signal suppression of the poorly ionized analytes by the easily ionized analytes could also cause the sensitivity differences between the analytes. In fact, the results obtained from the direct infusion of the same concentration of cocaine and benzoylecgonine, and

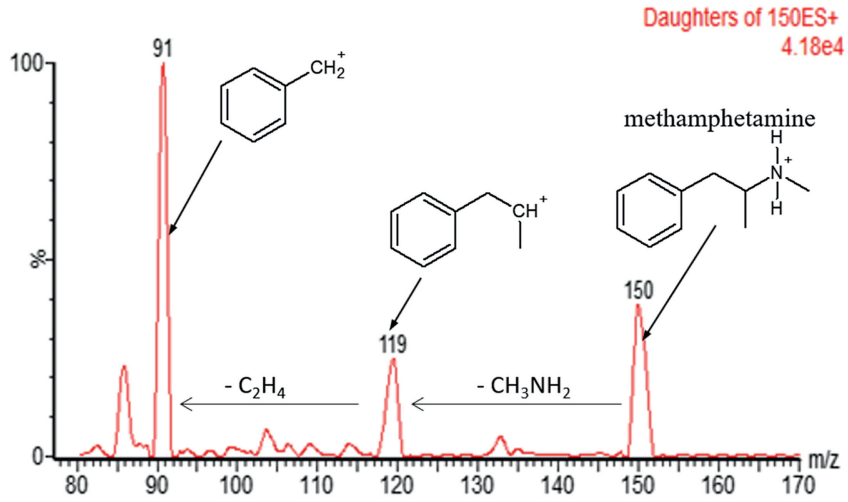

Fig. 8 – MS/MS spectrum obtained using 50 ng/mL methamphetamine in urine.

heroin and its metabolites showed some degree of signal suppression, with both the signals of cocaine and heroin higher than those of their metabolites. On the other hand, THC is a phenolic compound which is relatively hard to become protonated and thus tends to generate poorer signals. THC-COOH is a carboxylic acid which was supposed to generate better signals at negative ion mode. However, no obvious improvement was found with negative ion WT-ESI-MS in the present study.

3.3.4. Further confirmation of the drug identities by tandem mass spectrometry

Detection and quantitation of drugs-of-abuse using MRM are generally specific enough to identify the presence of drugs. The identities of detected drugs could be further confirmed by MS/MS analysis. For example, the identification of methamphetamine in urine is shown in Fig. 8. The presence of fragment ions of *m/z* 91 and *m/z* 119 could confirm the presence of methamphetamine.

4. Conclusions

Following our previous successful demonstration of WT-ESI-MS for rapid detection and quantitation of ketamine and norketaime [33], WT-ESI-MS has been extended to a rapid analysis of five more drugs in urine and oral fluid in this study. Analysis of one sample could be finished within minutes using WT-ESI-MS as only little sample preparation and no chromatographic separation was required. The good linearity and wide linear range for the targeted analytes enabled quantitative analysis using WT-ESI-MS. The accuracy and precision were generally satisfactory for quantitation of the targeted drugs except for morphine, THC, and THC-COOH. The LODs of the targeted analytes obtained by the present method were compared with the cut-off values of the three international guidelines. The detection of methamphetamine could fulfill the requirements of all the guidelines while detection of MDMA and cocaine could partially fulfill the requirements. Further improvement in sensitivity, such as by using surface-modified wooden tips, is required for the analysis of benzoylecgonine, heroin-related compounds, THC and THC-COOH. To conclude, the development of the present WT-ESI-MS could significantly reduce the time and labor required in drug analysis. Further development of this technique could be highly beneficial to the area of drug analysis as well as other fields demanding rapid and reliable detection and quantitation of molecules in complex mixtures.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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