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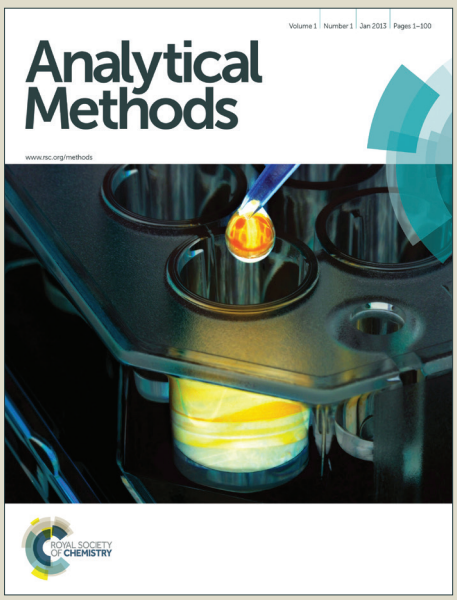

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Determination and quantitative analysis of designer drugs in human urine by liquid chromatography-mass spectrometry

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Abstract: A robust liquid chromatography-electrospray ionization-ion trap mass spectrometry (LC-ESI-ITMS) method was developed and utilized for the determination and quantitative analysis of four designer drugs in human urine. Designer drugs, including methcathinone (MC), 3, 4-methylenedioxymethcathinone (MDMC), methylenedioxy-pyrovalerone (MDPV) and 4'-methyl-α-pyrrolidinopropiophenone (MPPP) were determined by LC-ESI-ITMS under the optimal chromatographic separation conditions, and the precursor and major product ions of them were monitored in positive ion detective mode as m/z 164.0/146.0, m/z 208.0/190.1, m/z 276.2/205.0 and 218.1/147.0, respectively. Linear calibration curves showed good linearity in the range of 0.010 μg/mL to 5.00 μg/mL with coefficients of greater than 0.9988. The method was validated with the intra-day and inter-day precisions represented by relative standard deviation of less than 5.3% in human urine, and the recoveries from spiked urine samples varied from 79.5% to 94.6%. The obtained results indicated that the method was rapid, sensitive, selective, and it could be applied in the determination of designer drugs in forensic and clinically addict cases.

Keywords: liquid chromatography-mass spectrometry; designer drug; synthetic cathinones; urine

1 Introduction

Designer drugs, also named as synthetic drugs or novel psychoactive substances, they are synthesized to circumvent existing laws on controlled substances, and/or to enhance the pharmacological activities of already known drugs. Usually, they are made by modifying the molecular structures of existing drugs to varying degrees. Designer drugs have appeared on the illicit drug market and are available illicitly in tablet or power form in many countries, and they are sold as 'legal highs' or 'bath salts' in all regions. Designer drugs can be divided into nine classes according to the chemical structures, the typical favorite classed are synthetic cathinones and cannabinoids [1]. In order to aid law enforcement and also to understand what potential users may be subjected to, the analysis of these designer drugs and determination of their composition are necessary. Several analytical approaches have been applied in the determination of these designer drugs in pharmaceutical samples and biomaterials, including thin layer chromatography (TLC) [2], gas chromatography (GC) [3], gas chromatography-mass spectrometry (GC-MS) [4], high-performance liquid chromatography (HPLC) [5], liquid chromatography-mass spectrometry (LC-MS) [6] and capillary electrochromatography (CE) [7].

As a modern powerful analysis technology, LC-MS has been utilized widely in the research areas of life science, environmental science and so on [8]. The application has shown the superior advantages, such as higher sensitivity and superior selectivity comparing with other methods including HPLC, GC and GC-MS. Furthermore, the application of electrospray ionization ion trap mass spectrometry (ESI-ITMS) technique in LC-MS can lead to the acquirement of rich structural information from the analyses [9].

In the present study, a specific and sensitive method utilizing liquid chromatography-electrospray ionization ion trap mass spectrometry (LC-ESI-ITMS) was developed and applied for the simultaneous analysis of four designer drugs in human urine, the obtained results showed the advantages including rapidity, sensitivity and selectivity, but also shown the potential application in forensic and clinically addicted relevant cases of the approach mentioned here.

2 Experimental

2.1 Chemicals and reagent

Chromatography grade methanol, acetonitrile and analytical grade acetic acid were purchased from Shield Co., Ltd (Tianjin, China). Ammonium acetate was purchased from Dima Technology Inc.

(USA). Methcathinone (MC), 3, 4-methylenedioxymethcathinone (MDMC), methylenedioxy-pyrovalerone (MDPV) and 4'-methyl-α-pyrrolidinopropiophenone (MPPP) standards were all provided by Public Security Bureau of Nantong (Nantong, China) for research purposes. Human drug-free urine was obtained from volunteers and all of the experiments were performed in compliance with the relevant laws and institutional guidelines of National Police University of China, and also the institutional committee have been approved the experiments.

2.2 Preparation of standard solution and urine samples

Stock solutions of MC, MDMC, MDPV and MPPP were individually prepared in water with the concentration of 50.0 μg mL^{-1}. Calibration standard solutions of four designer drugs at nine concentrations were freshly prepared in the range of 0 to 10.00 μg mL^{-1} from the appropriate dilution of the stock solutions. Calibration curves were constructed using the detected peaks areas corresponding to the concentrations of the standards. Spiked urine samples with MC, MDMC, MDPV and MPPP were prepared in human drug-free urine and stored below 4℃ prior to use.

2.3 Treatment of urine samples

The samples were obtained by adding 100 μL acetonitrile in 100 μL of spiked urine samples, and then were centrifuged at 14 000 rpm for 10 min after vortex-mixed for 1 min, the supernatant were delivered, and an aliquot of 10 μL was injected to the LC-MS system for the determination and quantitative analysis.

2.4 LC/MS^n analysis

LC separation was accomplished with Finnigan Surveyor liquid chromatography system (San Jose, CA, USA) equipped with a Thermo Gold ODS column (150×2.1 mm, 5 μm). The mobile phase consisted of phase A (water, 10 mM ammonium acetate, adjusted to pH 4.50 with acetic acid) and B (methanol). The gradient program started with 10% B and held for 1 min, then increased to 90% B over 5 min, and held for 5 min. and then returned to the initial percentage over 0.5 min and maintained for 4.5 min, yielding a total run time of 15 min. The temperature of the column during analysis was maintained at 30℃. The flow rate was 0.2 mL min^{-1}.

MS analysis was performed on a LXQ ion trap mass spectrometer (Thermo Fisher, USA). The ion trap mass spectrometry was operated with positive electrospray ionization under full-scan MS, MS/MS and MS^3 modes. The flow rates of sheath gas, aux gas and sweep gas were 30.00 mL/min, 8.00 mL/min and 2.00 mL/min, respectively. The voltages of source, capillary and tube lens were

5.00 kv, 1.00 v and 5.00 v, respectively. The capillary temperature was 350℃. Data acquisition and instrument control were performed using Xcalibur software (Thermo Fisher, USA).

2.5 Accuracy and precision experiments

Accuracy and precision of the method referred by recovery and relative standard deviation (RSD) were both evaluated with the analyzing of the spiked urine samples. For measurement of intra-day precision and accuracy, urine samples with the concentrations of designer drugs at 0.2 μg mL^{-1}, 1.0 μg mL^{-1} and 3.0 μg mL^{-1} were prepared and analyzed three times within one day, respectively. The inter-day precision and accuracy was also determined with the same samples within three consecutive days.

3 Results and discussion

3.1 Optimization of LC-MS conditions

MC, MDMC, MDPV and MPPP are four typical designer drugs [10-13], belong to synthetic cathinones, and the structures of them are very similar as shown in Fig. 1. Based on our past work [14], ODS column was chosen as the separation column and other system parameters were also selected as mentioned in part 2.4. According to the corresponding reports [1, 15, 16], methanol and water (10 mM ammonium acetate, adjusted to pH 4.50 with acetic acid) were chosen as the mobile phases for the LC-ESI-ITMS analysis of four designer drugs mentioned here. Solvent gradient-elution program was established by comparing the peak resolution of four designer drugs obtained from different gradient-elution modes. The $[M+H]^+$ ions of them were chosen as parent ion for the fragmentation in MS/MS mode and the prominent ions in MS/MS spectra were chosen to fragmentate in MS^3 mode. The retention times of these designer drugs under the optimized gradient-elution conditions were 8.84 min, 9.75 min, 10.60 min and 10.37 min, respectively, which are shown in Fig. 2 and Table 1. The LC-MS^n spectra of four designer drugs are also listed in Fig.3, Fig. 4, Fig. 5, Fig. 6 and Table 1, respectively. It was noted that the peak intensity of the designer drugs was quite different even though the injected amount of each compound was similar, probably due to the difference in ionization efficiency. The $[M+H]^+$ ions of MC, MDMC, MDPV and MPPP were detected at m/z 164.0, m/z 208.0, m/z 276.2 and m/z 218.1, respectively. The most intensive ion peak of each analyte was selected as the quantitative ion in order to achieve the best sensitivity. The precursor and major product ions of the analytes were monitored in multiple reaction monitoring modes as follows: MC at m/z 164.0/146.0, MDMC at m/z 208.0/190.1, MDPV at m/z 276.2/205.0 and MPPP at m/z

218.1/147.0. Furthermore, the major product ions of four designer drugs monitored in MS^3 spectra were also obtained as m/z 164.0/146.0/131.1 for MC, 208.0/190.1/160.1 for MDMC, 276.2/205.0/174.9 for MDPV and 218.1/147.0/119.0 for MPPP, respectively, and the spectra could be benefit to the identifications of them in forensic science.

3.2 Calibration linearity and limit of detection

In order to study the linearity in response, spiking blank urine samples with known amounts of four designer drugs were prepared from 0 to 10.00 μg mL^{-1} at concentration levels: 0, 0.010, 0.050, 0.10, 0.50, 1.0, 2.5, 5.0 and 10.0 μg mL^{-1}, respectively. Calibration curves were constructed by analyzing the spiked urine samples. While the samples were treated and analyzed by LC-MS/MS, the obtained calibration curves of MC, MDMC, MDPV and MPPP exhibited good linearity in the ranges of 0.010 μg mL^{-1} to 5.00 μg mL^{-1} with coefficients from 0.9988 to 0.9993 as shown in Table 2. In order to estimate the limit of detection (LOD) and the limit of quantitation (LOQ), spiked urine samples at different concentrations were analyzed. The LODs and LOQs of four designer drugs developed in the present work are listed in Table 2, which were calculated on the basis of the chromatographic peak for which the signal-to-noise ratio was 3 (S/N=3) for qualitative analysis and 10 (S/N=10) for quantitative analysis, respectively. As shown in Table 2, LODs for MC, MDMC, MDPV and MPPP were 0.03 μg mL^{-1}, 0.02 μg mL^{-1}, 0.004 μg mL^{-1}, 0.02 μg mL^{-1}, and LOQs for them were 0.08 μg mL^{-1}, 0.05 μg mL^{-1}, 0.01 μg mL^{-1}, 0.05 μg mL^{-1} in spiked human urine, respectively.

As mentioned in illicit drugs case reports [17, 18], the concentrations of MDMC and MDPV in bio-samples of abusers were found below 4.3 μg mL^{-1} and beyond 0.060 μg mL^{-1}, furthermore, the concentrations of these designer drugs in urine were reported about 1.0 μg mL^{-1} of abusers in many cases, so the upper limit of quantification in our method was higher and the LOQ was below than the reports, therefore, the approach could be applied in the analysis of designer drugs in illicit cases.

3.3 Precision and repeatability

The precision referred by RSD was determined by analyzing spiked urine samples with different concentrations which were set with low, medium and high level of the calibration range as 0.2 μg mL^{-1}, 1.0 μg mL^{-1} and 3.0 μg mL^{-1} for all analytes. The intra-day precision was calculated by analyzing the samples within one day (n=3), while the inter-day precision was determined by analyzing the samples at the same concentrations in three consecutive days (n=9), and the results are listed in Table 3. The RSD from the intra-day study was generally lower than those from the

inter-day analysis which revealed from the results. Both RSDs were less than 5.3% as shown in table 3, indicating that the method has good precision and repeatability in the quantitative analysis of four designer drugs in human urine.

3.4 Recovery

While method accuracy was evaluated with the spiked urine samples at known levels of four designer drugs, the recovery experiments were also performed by analyzing the urine samples with LC-MS/MS analysis. The analysis data were obtained by comparing the average determined concentrations with the known spiked levels and are listed in Table 3. The recoveries varied from 79.5% to 94.6% were obtained from the analyzing of samples at low, medium and high concentrations, indicating that the method provided good accuracy for the analysis of designer drugs in human urine, and also revealed the potential superiorities in illicit drugs and metabolites analysis.

Above all, a fully validated analytical method for determination and quantitation of four designer drugs in human urine was presented. The validated examination results were acceptable and had shown the superiority of LC-ESI-ITMS, such as rapidity, accuracy, specificity and sensibility, and also the simple treatment procedure of urine sample.

4 Conclusions

In this study, the described LC-ESI-ITMS method provided determination and quantitative analysis of four designer drugs in human urine. The rapid, accurate, specific and sensitive analytical procedure was successfully applied for analysis of spiked human urine samples of MC, MDMC, MDPV and MPPP, and the experimental results showed that the potential advantages of this approach in the identification and quantitative analysis of designer drugs in forensic and clinically addicted relevant cases.

Acknowledgement

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Captions:

Fig. 1 Chemical structures of MC, MDMC, MDPV and MPPP

Fig. 2 LC-MS chromatograms of four designer drugs under the optimized gradient-elution conditions

Fig. 3 MS, MS/MS and MS^3 spectra of MC

(A) MS spectrum; (B) MS/MS spectrum; (C) MS^3 spectrum.

Fig. 4 MS, MS/MS and MS^3 spectra of MDMC

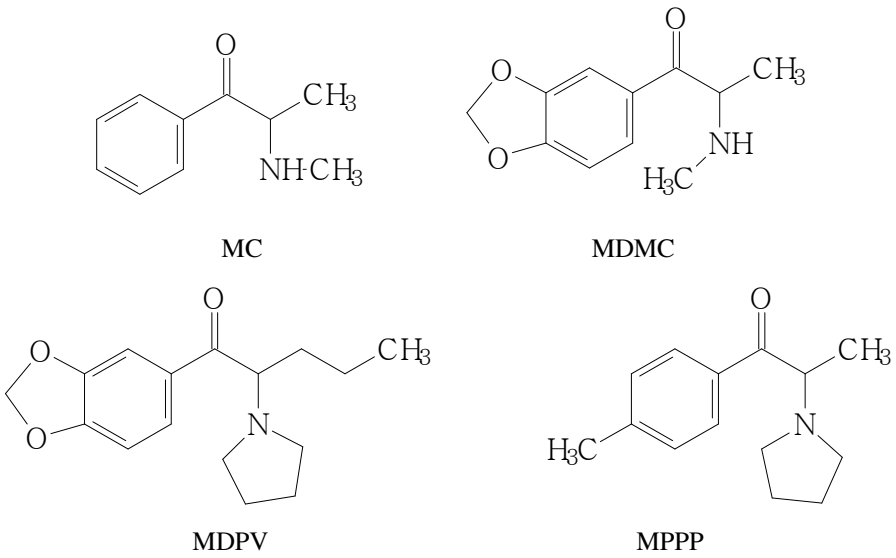
(A) MS spectrum; (B) MS/MS spectrum; (C) MS^3 spectrum.

Fig. 5 MS, MS/MS and MS^3 spectra of MDPV

(A) MS spectrum; (B) MS/MS spectrum; (C) MS^3 spectrum.

Fig. 6 MS, MS/MS and MS^3 spectra of MPPP

(A) MS spectrum; (B) MS/MS spectrum; (C) MS^3 spectrum.



MC MDMC

MDPV MPPP

Fig. 1

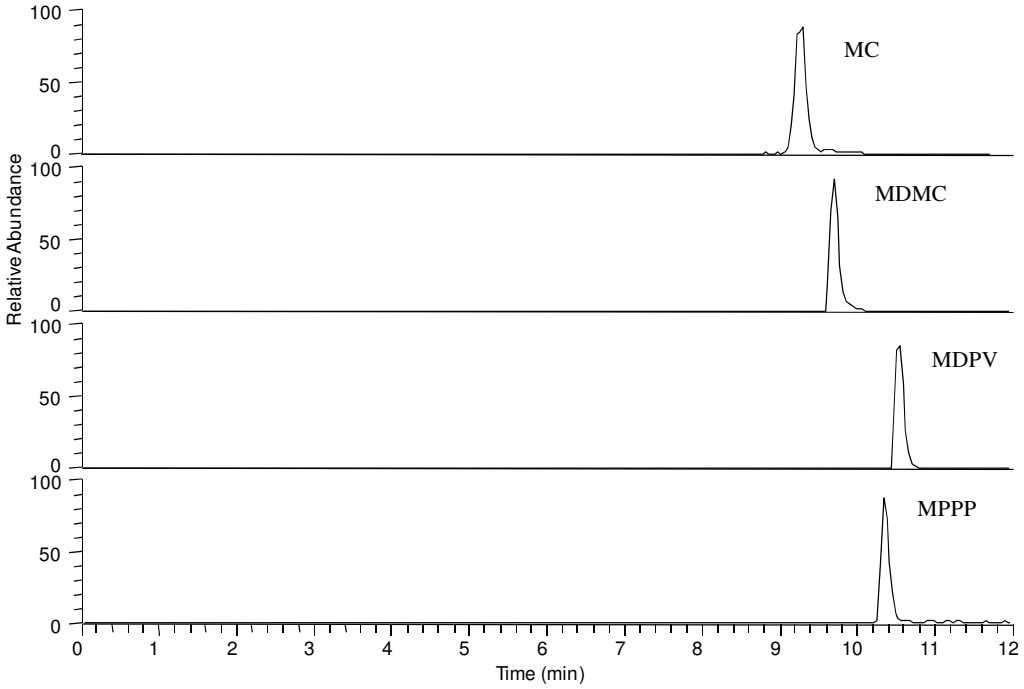


Fig. 2

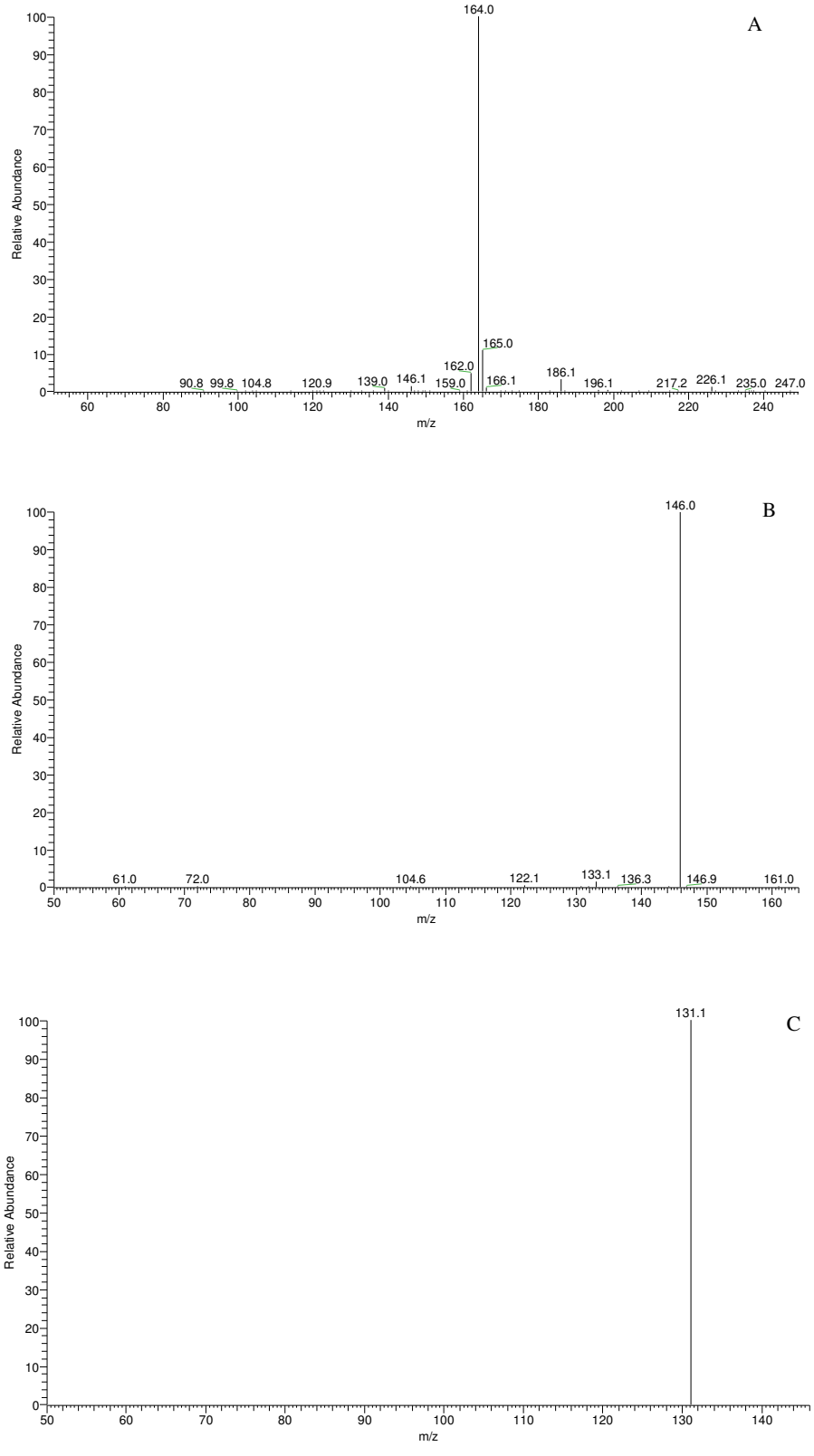


Fig. 3

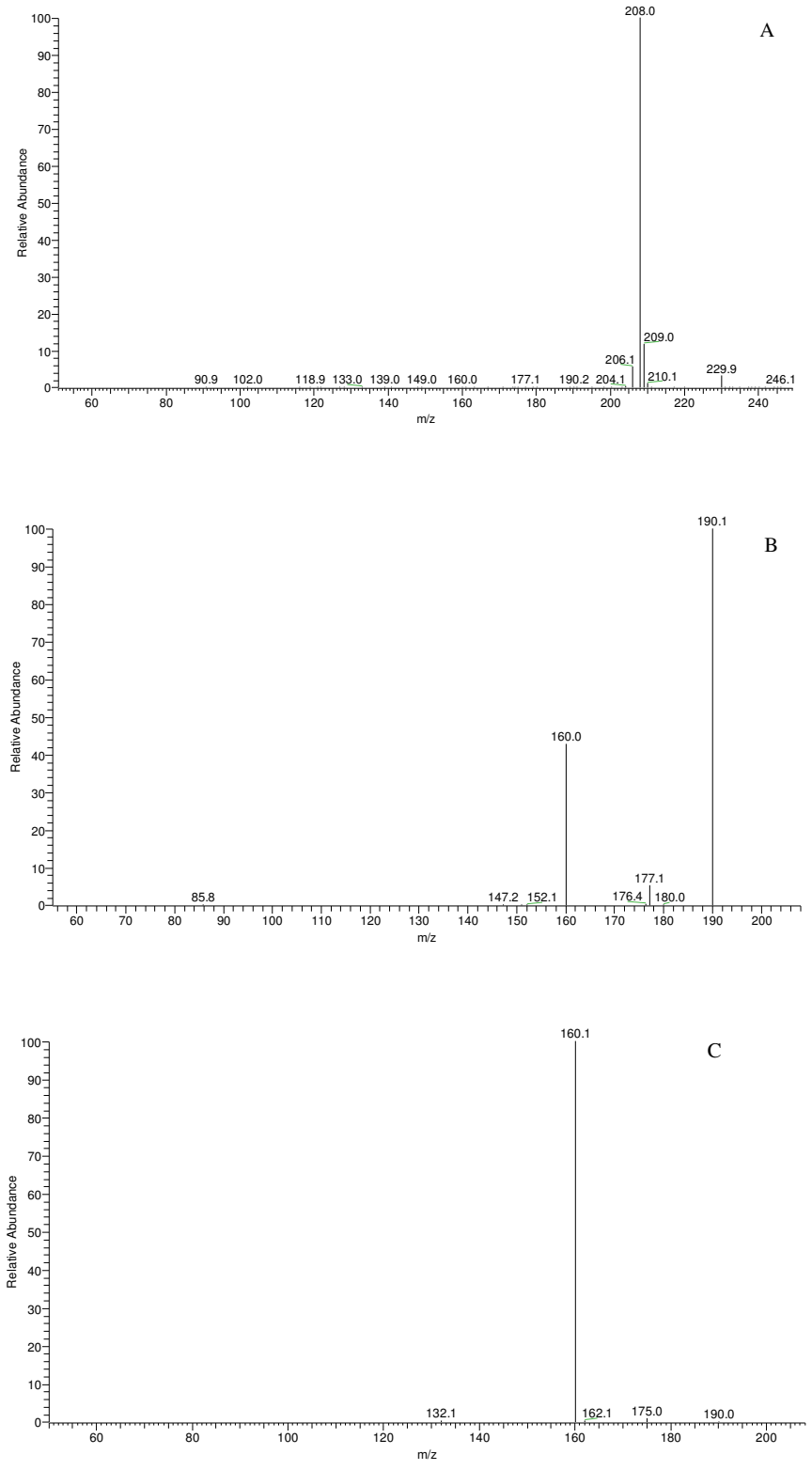


Fig. 4

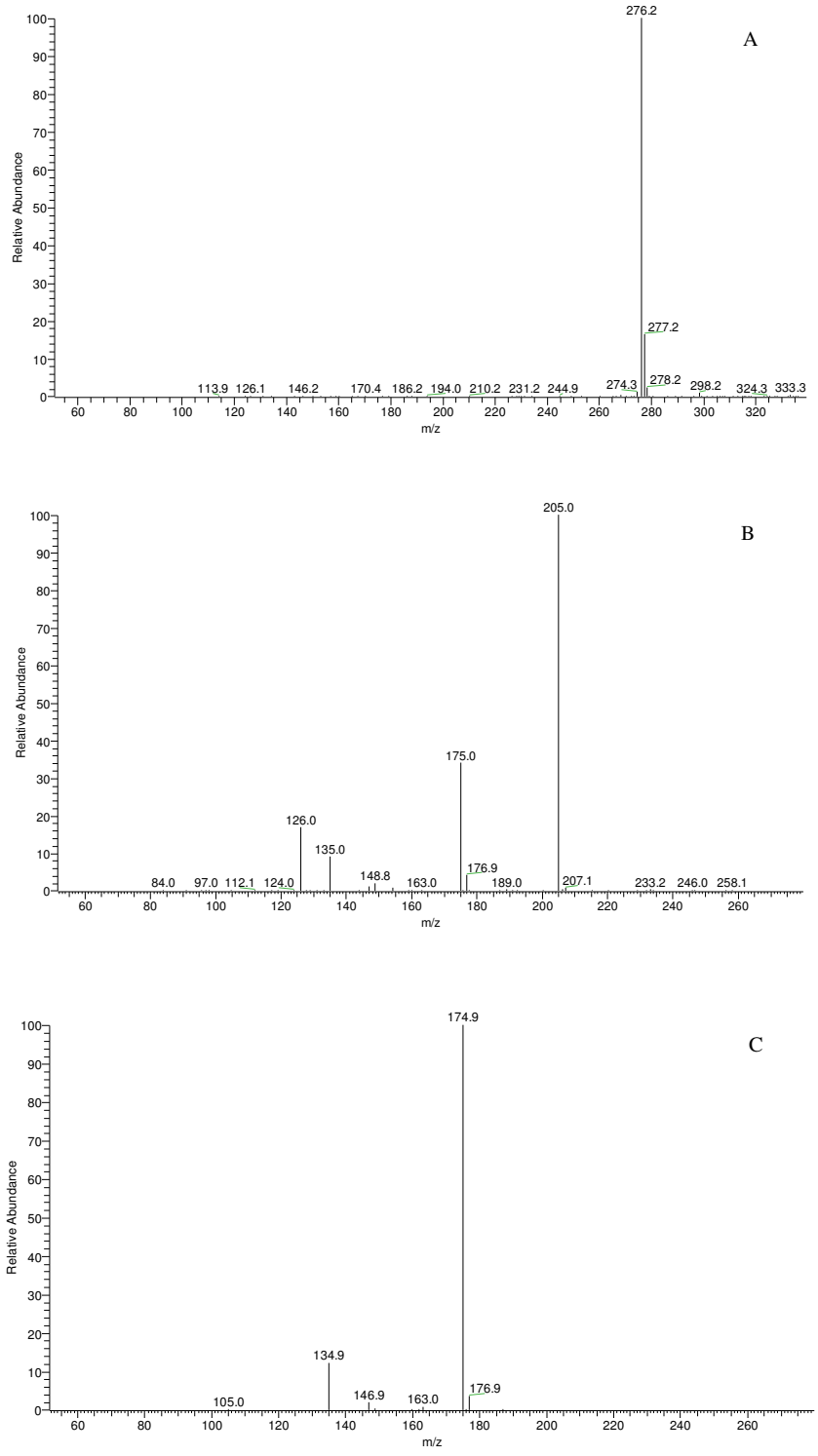


Fig. 5

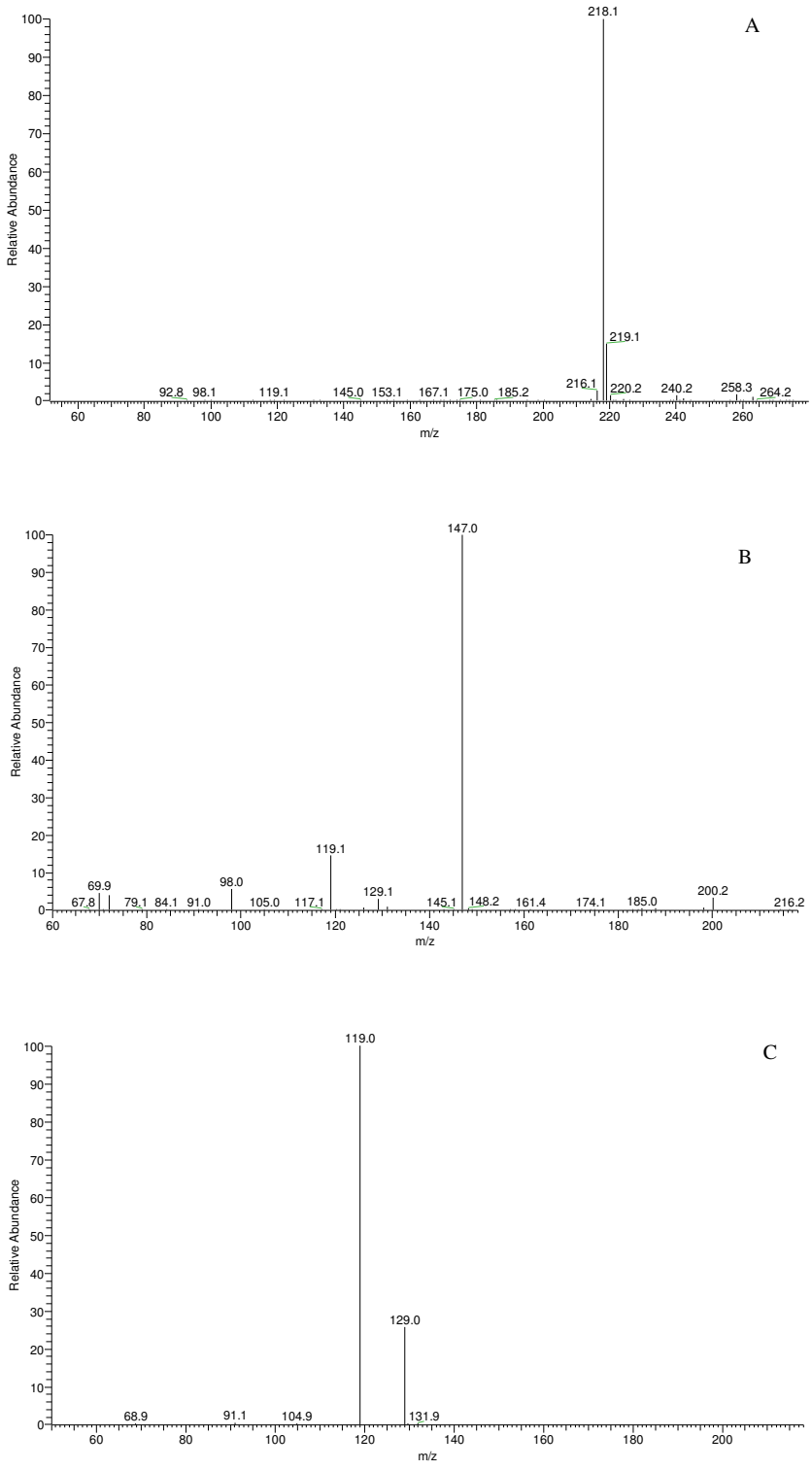


Fig. 6

Table 1 The exact mass, retention times and MS^n ion fragments of four desiger drugs

No	Name	Exact mass	Retention time (min)	$[M+H]^+$	MS/MS	MS^3
1	MC	163.1	8.84	164.0	146.0	131.1
2	MDMC	207.1	9.75	208.0	190.1	160.1
3	MDPV	275.2	10.60	276.2	205.0	174.9
4	MPPP	217.2	10.37	218.1	147.0	119.0

Table 2 Quantitative ions, linearity equations, coefficients, linearity ranges, LODs and LOQs of four designer drugs

Name	Quantitative ion	Linearity equation	Coefficients (r)	Linearity range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
MC	164.0/146.0	Y=23842X+1540.2	0.9991	0.08-5.00	0.03	0.08
MDMC	208.0/190.1	Y=15914X+1973.3	0.9988	0.05-5.00	0.02	0.05
MDPV	276.2/205.0	Y=18607X+1061.1	0.9993	0.01-5.00	0.004	0.01
MPPP	218.1/147.0	Y=20692X+1372.1	0.9989	0.05-5.00	0.02	0.05

Table 3 Precisions and recoveries of four designer drugs

Name	Spiked concentration ($\mu g\ mL^{-1}$)	Precision (RDS, %)		Recovery (%)
		Intra-day (n=3)	Inter-day (n=9)	
MC	0.20	4.9	5.3	79.5
	1.0	4.1	4.4	84.1
	3.0	3.5	3.8	85.3
MDMC	0.20	3.6	3.9	80.3
	1.0	3.3	3.6	86.4
	3.0	3.7	4.0	87.2
MDPV	0.20	3.5	3.6	90.0
	1.0	2.8	3.0	94.6
	3.0	3.1	3.3	93.8
MPPP	0.20	4.1	4.4	80.3
	1.0	3.8	4.0	88.7
	3.0	3.8	3.9	89.8