REPORT

Analysis of designer drugs in human blood using gas chromatography-mass spectrometry

Xueguo Chen* and Dan Zhao
Department of Forensic Chemistry, National Police University of China, Shenyang Liaoning, China

Abstract: A robust gas chromatography-mass spectrometry method was utilized in the qualitative and quantitative analysis of designer drugs in human blood. Designer drugs, including methcathinone, 3, 4-methylenedioxymethcathinone, 4'-methyl-α-pyrrolidinopropiophenone and methylenedioxy-pyrovalerone were simultaneously analyzed by gas chromatography-mass spectrometry. Liquid-liquid small volume extraction was employed in the pretreatment of human blood sample, and the experimental results showed that the method was validated with high extraction efficiency, low limits of detection and good linearity throughout the studied concentration ranges. Furthermore, the method not only exhibited good accuracy and precision in the determination of designer drugs in human blood, but also showed the potential of the approach in the determination of trace evidence in forensic science.

Keywords: Gas chromatography-mass spectrometry; designer drug; synthetic cathinones; human blood

INTRODUCTION

Designer drugs, also named as synthetic drugs or novel psychoactive substances, are synthesized in order to enhance the pharmacological activities of existing drugs (Leffler, 2014). Most of designer drugs are commonly made by modifying the molecular structures of already known drugs to varying degrees. They have appeared on the illicit drug market for a long time, and they are sold as 'recreational drugs' or 'bath salts' in tablet or power form in many countries (Smolianitski, 2014). Synthetic cathinones were one of the typical favorite classes of designer drugs in China. In order to understand what kind of potential people may be the abusers, the analysis of designer drugs is a necessary task. Several available analytical methods have been used in the determination of designer drugs, such as thin layer chromatography (Daeid, 2013), gas chromatography-mass spectrometry (GC-MS) (Lucas, 2000) and (Amini-Shirazi, 2010), liquid chromatography-mass spectrometry (Uralets, 2014), capillary electro chromatography (Aturki, 2014) and Fourier transform infrared spectroscopy (Abdel-Hay, 2014).

In this article, a simple and sensitive approach utilizing liquid-liquid small volume extraction coupled with GC-MS was applied in the qualitative and quantitative analysis of four designer drugs in human blood. The obtained results not only exhibited the good accuracy and high precision of the approach, and also showed the potential application for the trace evidence identification in forensic science.

**Corresponding author:* e-mail: dicpchenxg@hotmail.com

MATERIALS AND METHODS

Chemicals and reagent

Methcathinone (MC), 3, 4-methylenedioxymethcathinone (MDMC), 4'-methyl-α-pyrrolidinopropiophenone (MPPP) and methylenedioxy-pyrovalerone (MDPV) standards were all provided by Public Security Bureau of Nantong (Nantong, China) for research purposes and the purities were all above 95%. N, N-dimethylaniline, cyclohexane and other common chemicals solvents were all of analytical reagent grade and purchased from Guoyao Group Chemical Reagent Shenyang Co., Ltd (Shenyang, China).

Preparation of samples

Stock solutions of four designer drugs including MC, MDMC, MDPV and MPPP were individually prepared in deionized water with the concentration of 1.0mg/mL. The concentration of stock solution of internal standard N, N-dimethylaniline was 20μg/mL in methanol. Spiked blood samples with MC, MDMC, MDPV and MPPP were prepared by sequential dilution of stock solutions in human drug-free blood.

Treatment of blood samples

Fifty microliters Na_2CO_3-$NaHCO_3$ buffer (pH=10.8), 50mg NaCl, 0.5mL cyclohexane and 50μL N, N-dimethylaniline stocking solution were added to 5mL human blood in sequence and vortex-mixed for 3min, and then were centrifuged at 5000 rpm for 10 min, the supernatant were delivered and an aliquot of 1μL was injected to the GC-MS system for the qualitative and quantitative analysis.

GC-MS conditions

GC-MS analysis was performed using POLARIS Q gas chromatography-mass spectrometer (Thermo Fisher, USA) equipped with a manual injection and split liner. Separation was accomplished with a HP-5 MS capillary column (30m×0.25mm i.d., 0.25μm) and helium was chosen as the carrier gas at a constant flow rate of 1.0 mL/min. The column oven temperature program was optimized as following: the initial temperature was set as 60□, held for 1min and then increased to 280□at 20□/min, and then held at 280□ for 10 min. 1μL aliquot of sample was injected to the GC-MS system with a split ratio of 10:1. The temperatures of injector and GC interface were set as 280□ and 250□, respectively. Electron ionization (EI) ion source was utilized in the mass spectrometer, the energy was 70 eV and the ion source temperature was maintained at 250□. Acquisition mode was SCAN with the range of m/z 50~m/z 500. Xcalibur software (Thermo Fisher, USA) was using to accomplish the data acquisition and instrument control.

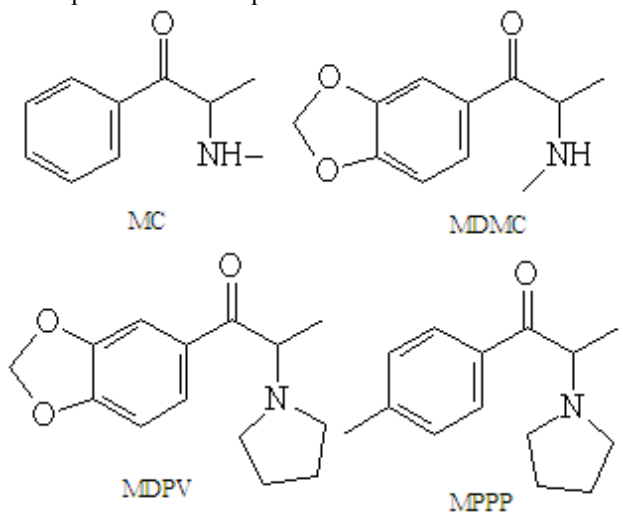

Fig. 1: Chemical structures of MC, MDMC, MDPV and MPPP

RESULTS

Optimization of the GC-MS analysis conditions

As typical designer drugs (Coppola, 2012) and (López-Arnau, 2013) and (Meyer, 2010) and (Springer, 2003), MC, MDMC, MDPV and MPPP belong to cathinones, and the structures of them are very similar as shown in Fig. 1. Column oven temperature program of the GC-MS analysis was optimized by comparing the peak resolutions of four designer drugs and international standard (IS) obtained from different programs according to the corresponding reports in literature (Leffler, 2014). The retention times of these four designer drugs and IS under the optimized column oven temperature program were 5.97min, 9.60min, 10.61min, 8.57min and 4.11min, respectively, and a typical chromatogram is presented in fig. 2.

Selection of extraction procedure

Generally, a sample pretreatment step is commonly necessary in the determination of analytes in blood with GC-MS, there are many methods including liquid-liquid extraction (Christner, 2014), solid-phase extraction (Saito, 2014) and solid phase micro extraction (Xiang, 2009) have been established and applied in the approach. Small volume liquid-liquid extraction was proved to be simple, rapid and sparing of solvent and experimental time compared to regular volume liquid-liquid extraction, and it has been employed in the analysis of drugs (Meng, 2008), and it was employed in our study. As we know, ethyl ethanoate, benzene, cyclohexane, toluene are commonly extracting solvents utilized in the extraction procedure, they were examined in our study and the results showed that the extraction efficiency of cyclohexane was higher than others, so it was employed as the extractant in the LLE of designer drugs in human blood. Furthermore, the usage of cyclohexane, salting-out effect, sample pH and extraction time were all tested and optimized by comparing the extraction efficiency of these designer drugs in human blood, thus the pretreatment procedure of the blood was established.

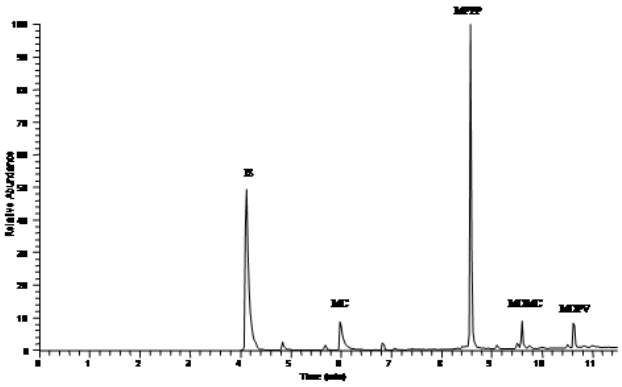

Fig. 2: GC-MS chromatograms of four designer drugs

DISCUSSION

Characteristic of designer drugs with GC-MS

In order to determine the characteristic mass fragments, primary EI mass spectra and product spectra of four designer drugs were recorded in full scan mode with GC-MS. The character fragment ions could be observed in the EI spectra of MC, MDMC, MPPP and MDPV, respectively. Two of the fragment ions were chosen for the confirmation of designer drugs, which are shown in table 1. Furthermore, the postulated fragment ions of these four designer drugs were also benefit to the qualities analysis of designer drugs in forensic science.

Moreover, calibration linearity of the method was performed by analyzing MC, MDMC, MPPP and MDPV with different concentrations in human blood using GC-MS and the calibration curves were obtained by plotting the peak-areas of q designer drugs against the concentrations of them in human blood. Good linearities

were obtained in the range of 0.01-5.0μg/mL and the correlation coefficients were greater than 0.9912. Moreover, limit of detection (LOD) and limit of quantitation (LOQ) of four designer drugs were estimated by analyzing the spiked samples at different concentrations and the results are listed in Table 2. Furthermore, the recoveries of MC, MDMC, MPPP and MDPV were also assessed by spiking human drug-free blood with the analytes at 0.5μg/mL, 1.0μg/mL and 2.0μg/mL, respectively, and the results are shown in Table 3. Data in Table 3 shows that the recoveries of these four designer drugs ranged from 76.3% to 93.6%.

Table 3: Recoveries of four designer drugs

Name	Spiked concentration (μg/mL)	Recovery (%)
	0.20	76.3
MC	1.0	81.4
	3.0	82.8
	0.20	77.5
MDMC	1.0	82.8
	3.0	86.2
	0.20	89.2
MDPV	1.0	92.3
	3.0	93.6
	0.20	78.1
MPPP	1.0	84.9
	3.0	86.9

CONCLUSIONS

A reliable analytical method based on GC-MS was established for the simultaneous determination of four designer drugs in human blood. Convenient sample preparation was achieved with small volume liquid-liquid extraction procedure, and satisfactory results were obtained by the analysis of spiking human drug-free blood samples with MC, MDMC, MDPV and MPPP. Moreover, the application showed the potential advantages of this method in evidence identification of forensic science.

ACKNOWLEDGEMENT

The financial supports of the Faculty Research Grant from Key Laboratory of Evidence Science of Ministry of Education (No. 2014KFKT05) and Ministry of public security (No. 2015JSYJB09) in this study are acknowledged.

REFERENCES

Abdel-Hay KM, DeRuiter J and Clark CR (2014). Regioisomeric bromodimethoxy benzyl piperazines related to the designer substance 4-bromo-2,5-dimethoxybenzylpiperazine: GC-MS and FTIR analysis. *Forensic Sci. Int.*, **240**: 126-36.

Amini-Shirazi N, Ahmadkhaniha R, Shadnia S, Ghahremani MH, Abdollahi M, Dadgar A and Kebriaeezadeh A (2010). Determination of VPA and its two important metabolites in iranian overdosed patients. *Int. J. Pharm.,* **6**(6): 854-862.

Aturki Z, Schmid MG, Chankvetadze B and Fanali S (2014). Enantiomeric separation of new cathinone derivatives designer drugs by capillary electro chromatography using a chiral stationary phase, based on amylose tris (5-chloro-2-methylphenylcarbamate). *Electrophoresis*, **35**(21-22): 3242-3249.

Christner SM, Parise RA, Levine ED, Rizvi NA, Gounder MM and Beumer JH (2014). Quantitative method for the determination of iso-fludelone (KOS-1803) in human plasma by LC-MS/MS. *J. Pharm. Biomed. Anal.*, **100**: 199-204.

Coppola M and Mondola R (2012). Synthetic cathinones: chemistry, pharmacology and toxicology of a new class of designer drugs of abuse marketed as “bath salts” or “plant food”. *Toxico. Letters*, **211**: 144-149.

Daeid NN, Savage KA, Ramsay D, Holland C and Sutcliffe OB (2013). Development of gas chromatography-mass spectrometry (GC-MS) and other rapid screening methods for the analysis of 16 ‘legal high’ cathinone derivatives. *Sci. Justice*, **54**(1): 22-31.

Leffler AM, Smith PB, Armas A and Dorman FL (2014). The analytical investigation of synthetic street drugs containing cathinone analogs. *Forensic Sci. Int.*, **234**: 50-56.

López-Arnau R, Martínez-Clemente J, Carbó MI, Pubill D, Escubedo E and Camarasa J (2013). An integrated pharmacokinetic and pharmacodynamic study of a new drug of abuse, methylone, a synthetic cathinone sold as “bath salts”. *Pro. Neuro-psychoph.*, **45**: 64-72.

Lucas ACS, Bermejo AM, Tabernero MJ and Fernandez P (2000). Use of solid-phase micro extraction (SPME) for the determination of methadone and EDDP in human hair by GC-MS. *Forensic sci. int.*, **107**(1-3): 225-232.

Meng PJ, Wang YY and Zhu D (2008). Small volume liquid extraction of amphetamines from bio-samples and GC/MS analysis. *Chin J. Appl. Chem.*, **25**(12): 1449-1455.

Meyer MR, Du P, Schuster F and Maurer HH (2010). Studies on the metabolism of the α-pyrrolidinophenone designer drug methylenedioxy-pyrovalerone (MDPV) in rat and human blood and human livermicrosomes using GC-MS and LC-high-resolution MS and its detectability in blood by GC-MS. *J. Mass Spectrom.*, **45**: 1426-1442.

Saito K, Kikuchi Y and Saito R (2014). Solid-phase dispersive extraction method for analysis of benzodiazepine drugs in serum and blood samples. *J. Pharm. Biomed. Anal.*, **100**: 28-32.

Smolianitski E, Wolf E and Almog J (2014). Proactive

forensic science: A novel class of cathinone precursors. *Forensic Sci. Int.*, **242**: 219-227.

Springer D, Fritschi G and Maurer HH (2003). Metabolism of the new designer drug α-pyrrolidinopropiophenone (PPP) and the toxicological detection of PPP and 4'-methyl-α-pyrrolidinopropiophenone (MPPP) studied in rat blood using gas chromatography-mass spectrometry. *J. Chromatogr. B.*, **796**: 253-266.

Uralets V, Rana S, Morgan S and Ross W (2014). Testing for designer stimulants: Metabolic profiles of 16 synthetic cathinones excreted free in human blood. *J. Anal. Toxicol.*, **38**(5): 233-241.

Xiang P, Shen M and Zhuo XY (2009). LC-MS and application in the analysis of medicine and abused drugs, Shanghai Science and Technology Press, Shanghai, China, pp.182-188