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Simultaneous determination of four designer drugs and their major metabolites by liquid chromatography–mass spectrometry

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ABSTRACT

A sensitive liquid chromatography–electrospray ionization-ion trap mass spectrometry (LC–ESI-ITMS) method was utilized for the simultaneous analysis of four designer drugs and their in vitro metabolites in rat liver microsome S_9 fraction. Four designer drugs, including methcathinone (MC), 3,4-methylenedioxymethcathinone (MDMC), 3,4-methylenedioxy-pyrovalerone (MDPV) and 4′-methyl-α-pyrrolidinopropiophenone (MPPP), were individually incubated with rat liver microsome S_9 fraction, and the incubation mixtures were pooled together and analyzed by LC–ESI-ITMS simultaneously. Besides four designer drugs, five of their main metabolites were identified via the analysis of protonated molecules and tandem mass spectrometry data. Meanwhile, the quantification analysis of four designer drugs in rat liver microsome S_9 fraction was performed, the calibration curves showed good linearity in the range of 0.01–5.0 μg/mL and the detection limits were below 0.03 μg/mL with RSDs less than 5.9% and recovery ratios above 77.4%. The experimental results not only showed that these designer drugs could be easily metabolized in rat liver microsome, and also displayed the superiorities of the method including time and cost saving, high efficiency, sensitivity and selectivity. The studies in this study indicated that the approach could be applied in the determination of illicit drugs and their metabolites in medical, pharmaceutical and forensic investigations.

1. Introduction

Designer drugs, also named as synthetic drugs or novel psychoactive substances, have appeared on the illicit drug market and are available illicitly in tablet or powder form in many countries. They are sold as 'legal highs' or 'bath salts' in all regions. Designer drugs can be divided into nine classes according to the structures, and the most popular categories are synthetic cathinones and cannabinoids [1]. In order to aid law enforcement and to understand what potential users may be subjected to, the analysis of designer drugs and the determination of their composition are necessary. Several modern analytical techniques have been applied in the determination of designer drugs in pharmaceutical samples and biomaterials, including thin layer chromatography [2], gas chromatography [3], gas chromatography–mass spectrometry [4], high-performance liquid chromatography [5], liquid chromatography–mass spectrometry (LC–MS) [6] and capillary electrochromatography [7]. Among these analysis technologies, LC–MS has been widely utilized in the research areas of life science, environmental science and forensic science [8,9] owe to its high sensitivity and superior selectivity [10].

In the present study, a specific and sensitive method utilizing LC–ESI-ITMS was applied for the simultaneous analysis of four designer drugs and their major metabolites in rat liver microsome S_9 fraction after in vitro metabolism study in order to show the advantages and potential applications of LC–MS in the identification of forensic evidence.

2. Experimental

2.1. Chemicals and reagent

Chromatography grade methanol, acetonitrile were purchased from Shield Co., Ltd. (Tianjin, China). Analytical reagent grade acetic acid and ammonium acetate were purchased from Guoyao Group Chemical Reagent Shenyang Co., Ltd. (Shenyang, China). Methcathinone (MC) and 3,4-methylenedioxy-pyrovalerone (MDPV) were purchased from Sigma–Aldrich (St. Louis, MO, USA), 3,4-methylenedioxymethcathinone (MDMC) and 4′-methyl-α-pyrrolidinopropiophenone (MPPP) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All the standards of designer drugs were

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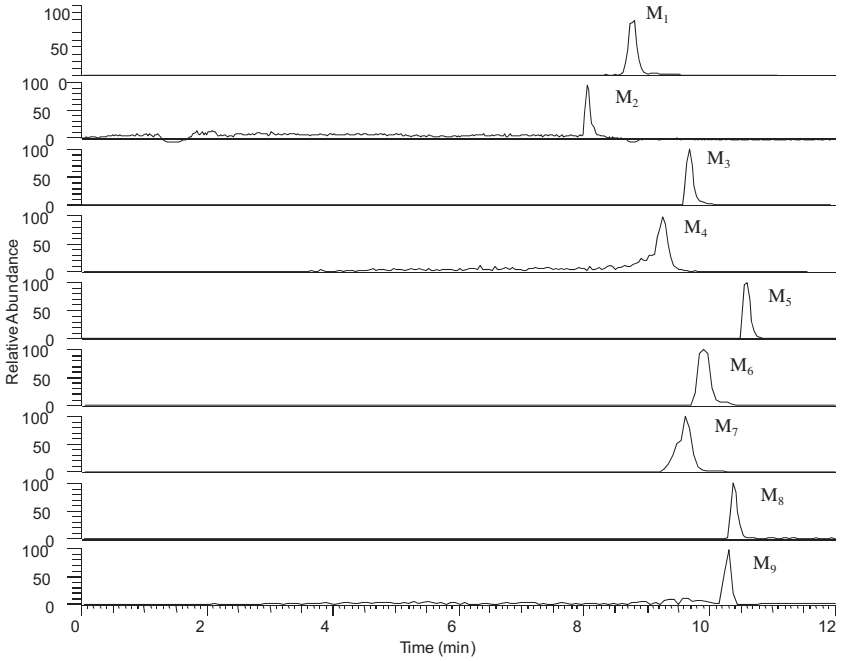

Fig. 1. LC–MS chromatograms of four designer drugs and their metabolites under the optimized gradient-elution conditions. Peaks identification: M_1. MC, M_2. Cathinone, M_3. MDMC, M_4. 3-OH-4-MeO-MC, M_5. MDPV, M_6. Methyl catechol pyrovalerone, M_7. Catechol pyrovalerone, M_8. MPPP, M_9. Oxo-MPPP.

only used for research purpose. β-Nicotinamide-adenine dinucleotide phosphate reduced form (β-NADPH) was obtained from iPhase Pharma Services (Beijing, China). Rat liver microsome S_9 fraction was prepared from male Sprague Dawley (S.D.) rats (about 8 weeks old and bought from Laboratory Animal Services Center of Liaoning University of Traditional Chinese Medicine). The protein concentration of rat liver microsome S_9 fraction was determined by Bradford method [11] and the concentration of cytochrome P_{450} was obtained with Omura method [12].

2.2. In vitro incubation and sample extraction

Individual stock solutions of MC, MDMC, MDPV and MPPP were prepared at a concentration of 50.0 μg/mL. 20 μL of stock solution of MC was added to 200 μL rat liver microsome S_9 fraction solution. After shaken 5 min in water bath at 37 °C, 5 mg NADPH was added. The reaction was quenched by adding 200 μL of acetonitrile 120 min later, and centrifuged at 14,000 rpm for 20 min. The same procedure was repeated except the drug was changed to MDMC, MDPV or MPPP. Finally, 50 μL of the supernatants were removed from four resulted solution after incubation, and pooled together as experimental samples. A blank sample was also gained after dealing rat liver microsome S_9 fraction solution with no drug added following the above procedure. An aliquot (20 μL) of each samples was injected into the LC–MS system.

2.3. LC/MS^n analysis

LC separation was accomplished with Finnigan Surveyor liquid chromatography system (Thermo Fisher, USA). A Thermo Gold ODS column (150 × 2.1 mm, 5 μm) was used, the mobile phases were consisted of phase A (water, 10 mM ammonium acetate, 0.1% acetic acid) and B (methanol). The gradient-elution program started with 10% B and held for 1 min, then changed to 90% B within 5 min, and held for another 8 min. The flow rate was 0.2 mL/min.

MS analysis was performed on a LXQ ion trap mass spectrometer (Thermo Fisher, USA). Positive ion mode was utilized. 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 °C. Data acquisition and instrument control were performed using Xcalibur software (Thermo Fisher, USA).

3. Results and discussion

3.1. Optimization of LC–MS conditions

Mobile phases were chosen according to the structure of drugs and previous report [1,13,14]. Solvent gradient-elution program was optimized by comparing the peak resolutions of four designer drugs obtained from different gradient-elution modes. The $[M+H]^+$ ions of drugs were chosen as parent ions for the fragmentation in MS/MS mode and the prominent ions in MS/MS spectrum were chosen to fragmentize in MS^3 mode. The retention times and tandem mass spectrometry (MS^n) ion fragments of four designer drugs under the optimized gradient-elution conditions are shown in Fig. 1 and Table 1.

3.2. Method validation

In our study, LC–MS/MS was utilized for the quantitative analysis of MC, MDMC, MDPV and MPPP in rat liver microsome S_9 fraction. The base fragment ions of MC (*m/z* 146.1), MDMC (*m/z* 190.1), MDPV (*m/z* 205.0) and MPPP (*m/z* 147.0) were chosen as quantitation ions, respectively. Selectivity of the method was tested by comparing the chromatograms of blank sample with experimental samples. The obtained results showed that there was no background interference to the analysis of four designer drugs under the optimal LC–MS/MS conditions.

In order to study the linearity of response, four designer drugs were individually spiked into blank sample of rat liver microsome S_9 fraction at nine concentration levels of 0, 0.01, 0.05, 0.10, 0.50, 1.00, 2.50, 5.00 and 10.00 μg/mL. The obtained calibration curves of MC, MDMC, MDPV and MPPP all exhibited good linearity in the range of 0.01–5.00 μg/mL, the linearity equations, coefficients and