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The establishment of a highly sensitive method in detecting ketamine and norketamine simultaneously in human hairs by HPLC-Chip–MS/MS

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ABSTRACT

An effective way to reveal the history of drug abuse is to determine the parental drug and its metabolites in hair. Here, a quantitative HPLC-Chip–MS/MS method was developed for simultaneous measurement of ketamine and its metabolite norketamine in human hair. Ketamine and norketamine were extracted from hair by acid hydrolysis, and then enriched by organic solvent extraction. The chromatographic separation was achieved in 15 min, with the drug identification and quantification by a tandem mass spectrometer. The linear regression analysis was calibrated by deuterated internal standards with a R^2 of over 0.996. The limit of detection (LOD) and the limit of quantification (LOQ) for ketamine and norketamine were 0.5 and 1 pg/mg of hair, respectively. The standard curves were linear from the value of LOQ up to 100 pg/mg of hair. The validation parameters including selectivity, accuracy, precision, stability and matrix effect were also determined. In conclusion, this method was able to reveal the present of ketamine and norketamine with less hair from the drug abusers, and which had the sensitivity of ~1000-fold higher than the conventional method. In addition, the amount of ketamine and norketamine being detected in different hair segments would be useful in revealing the historical record of ketamine uptake in the drug abusers.

1. Introduction

Drug abuse has become one of the major social problems in all countries. To cope with this crisis, an accurate determination of drugs in the biological specimen collected from drug abusers becomes a critical factor. The unreliable drug testing results not only might be contested in the court, but also could lead to unjustified legal consequences for the defendant, or more seriously that might cause a wrong treatment for the clients [1]. Several human specimens including urine, hair, blood, sweat and saliva have been used in determining the residues of abused drugs. Among them, urine, hair and saliva in drug testing have frequently been reported. The urine testing is the most popular method to monitor the drug abuse, which however provides only a short-term historical record of the drug exposure (less than 7 days) [2]. More important, the specimen could easily be adulterated [3]. The collection of saliva is perceived as less invasive and susceptible to adulteration as compared to urine specimen. However, the amount of drug and its metabolites are lower in saliva than in urine, which therefore demands a much higher sensitivity of the analytic apparatus. Similar to the urine testing, the testing on saliva reveals a short-window of drug abused record. Therefore, the drug testing from human saliva is easily to lead imprecise results [4]. In contrast to urine and saliva testing, hair specimen supplies a longer detection window (more than 1 year, if the hair is longer than 12 cm as the average hair growth rate is ~1 cm per month). Even though individual variation in terms of the hair growth is commonly found, the hair-drug testing still enables retrospective investigation of the past drug consumption. In addition, hair can be easily obtained and difficult to adulterate, and which could be stored and transported without specific precautions owing to its stability [5]. Another advantage of hair-drug testing is that the segmental hair analysis might help to determine the time of drug exposure [6]. Furthermore, hair contains a relatively high parental drug to metabolite ratio, i.e. it is much easier to identify the drug abusers.

Gas chromatography–mass spectrometry (GC–MS) is currently still the standard technique in anti-doping analysis for hair-drug testing, mainly due to its robustness and high level of standardization [7]. However, liquid chromatography–mass spectrometry (LC–MS) has been widely employed in different analyses in recent years. By comparing to GC–MS, the greatest advantage of LC–MS is that the polar compounds could be chromatographically separat-

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ed, and no chemical derivatization is needed, which simplifies the sample preparation. However, no matter the GC–MS or LC–MS method, the amount of hair sample required is always about 20 mg to 50 mg. Unfortunately, such an amount of hair would greatly limit high-throughput applications and increase the time spent in cutting and washing the hair samples. Requiring smaller sample size would undoubtedly increase the efficiency of sample preparation. In order to meet this requirement, the recent utilization of nano-HPLC technology (nano-LC) allows the analysis with high sensitivity on picogram level or less. However, this technique has been involved mainly for protein analysis [8,9], and no application of HPLC-Chip on the hair-drug testing has been reported.

Ketamine, known as "K" or "Special-K" or "Kit-Kat" in Hong Kong, has been used as an anesthetic primarily in veterinary medicine and in some short-term surgical procedures in humans since its discovery in 1961. Norketamine is the major metabolite of ketamine *in vivo* [10]. Ketamine, an antagonist of NMDA receptor, produces post-hypnotic emergence reactions such as prolonged hallucination and delirium, which therefore has led to its abuse as a recreational drug [11]. Ketamine misuse has been reported in many countries [12–15]. The drug abuse is a serious problem in Hong Kong, and indeed ketamine is the most commonly abused drug. Displayed by the statistical data from the Narcotics Division of Security Bureau of Hong Kong Government, over 68% of drug abusers were taking ketamine in 2008 and 2009. To be shocked, the percentage of ketamine abuser, under 21 of age, was up to 83.5% in 2009. After the administration of ketamine, this drug is rapidly degraded in hours: the rapid degradation hinders the detection of this drug in urine specimen by those antibody-coated test kits, or even determined by GC–MS and/or LC–MS/MS [16,17]. For hair specimen, GC–MS and LC–MS/MS have been employed in ketamine testing: the detection limit was ranged from 0.1 ng/mg to 1.7 ng/mg of hair, respectively [18–20]. Because of this relative low sensitivity, relative large amounts of hair (about 50 mg) have to be used for such analysis [21–23]. Here, a HPLC-Chip–MS/MS system was developed for the hair-drug testing. This novel method possesses an extremely high sensitivity of femtogram detection, and which therefore requires in principle only 2 mg of hair.

2. Experimental

2.1. Chemicals

Ketamine dried powder was purchased from Sigma–Aldrich (St. Louis, MO). Methanolic solutions of norketamine HCl (1 mg/mL), ketamine-D_4 (0.1 mg/mL), norketamine-D_4 HCl were purchased from Cerilliant (Round Rock, TX). All of these standards have a purity of over 99% based on the HPLC profile, UV and MS data. Their chemical structures are shown in Fig. 1. HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany), formic acid was purchased from Riedel-de Haën International Ltd. (Darmstadt, Germany). Methanol used for extraction was purchased from Merck Company. Ultra pure water was prepared from a Milli-Q purification system Millipore S.A. (Molsheim, France). Other reagents used here were of analytical grade.

2.2. Human hair specimens

Human hair specimens were collected from the drug abusers in Shenzhen Detoxification Institute (Shenzhen, China). Hair was cut with round-point scissors from the vertex posterior of the scalp. The segments corresponding to a 3-cm long from the root end were preserved at room temperature until analysis. Blank hair was obtained from healthy donors with no history of drug abuse. The collection of human hair was approved by Human Participants Research Panel at the Hong Kong University of Science and Technology.

2.3. Instrumentation

All experiments were carried out by using an Agilent 6410B-triple quadrupole mass spectrometer (QQQ-MS/MS) with an HPLC-Chip Cube interface (Agilent Technologies, Waldbronn, Germany) coupled with a nano-LC. Liquid chromatography was performed by using an Agilent 1200 instrument consisting of a 1200 nanoflow pump with a degasser, a 1200 capillary pump with a degasser and a 1200 thermostat microwell-plate sampler. The following components were integrated onto the HPLC polymeric Chip (G4240-63001-Agilent Technologies): a 25 mm, 500 nL enrichment column packed with ZORBAX 80 SB-C18 with 5 μm particle size and a 150 mm × 75 μm separation column packed with ZORBAX 80 SB-C18 with 5 μm particle size. The HPLC polymeric Chip was inserted into the HPLC-Chip–MS/

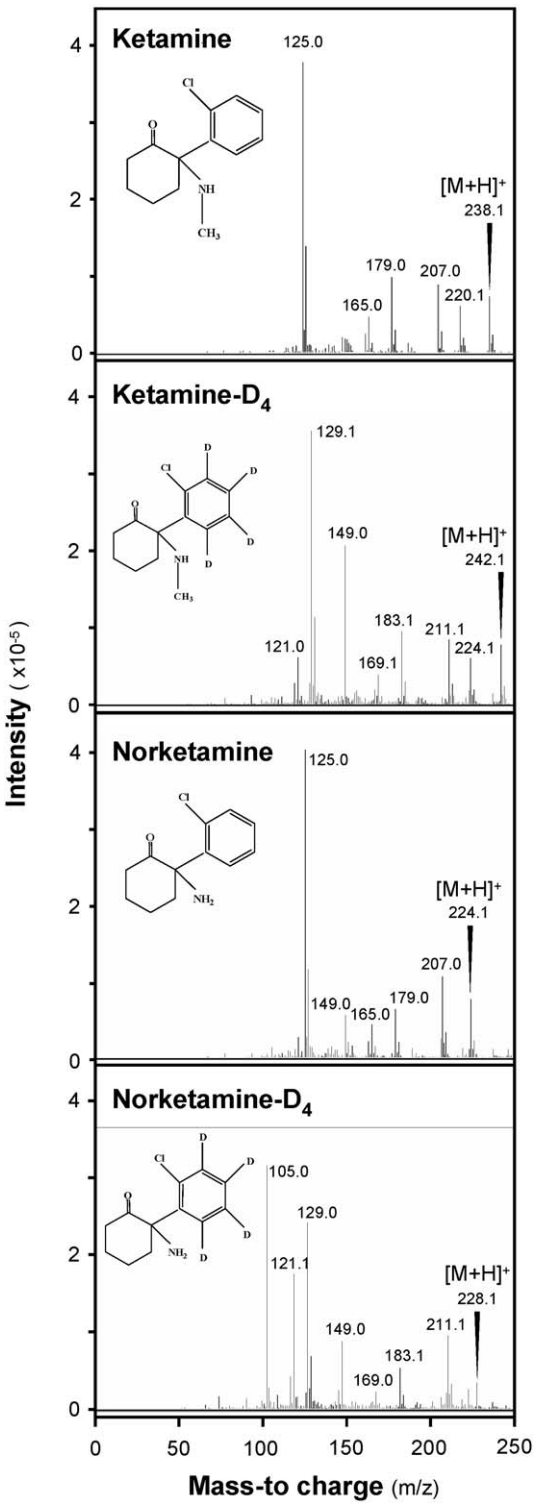

Fig. 1. Mass spectra of ketamine, norketamine, ketamine-D_4, norketamine-D_4. The MS/MS spectrum was acquired by the product ion scan mode firstly. Then, the two product ions were selected for the further multiple reaction monitoring (MRM) scan mode. These ions were ketamine at 125 and 179, norketamine at 125 and 207, ketamine-D_4 at 129 and 224, and norketamine-D_4 at 129 and 211. The optimized precursor ion, fragmentor, collision energy and product ion are listed as in Table 1.