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GC–MS quantification of ketamine, norketamine, and dehydronorketamine in urine specimens and comparative study using ELISA as the preliminary test methodology

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Abstract

An automated solid-phase extraction procedure combined with the gas chromatography–mass spectrometry (GC–MS) methodology, without derivatization, has been developed for the determination of ketamine (K), norketamine (NK), and dehydronorketamine (DHNK) in urine. The analytical approach is simple and rapid, yet reliable, achieving good linearity ($r^2 > 0.999$ over the concentration range of 30 to 1000 ng/mL), sensitivity (limits of quantification = 15, 10, and 20 ng/mL for K, NK, and DHNK, respectively), accuracy (90–104%), and precision (RSD < 8.1%) for all analytes. Two hundred and six urine specimens collected from suspected drug users were analyzed by this protocol and also screened by Neogen ELISA method to evaluate the efficiency as well as the compatibility of these two methods. Neogen ELISA showed high efficiency (98.1%), high sensitivity (90.9%), high specificity (98.9%), low false-positive rate (1.1%), and moderate false-negative rate (9.1%), adopting 10 ng/mL K as the cutoff. Neogen ELISA screening followed by GC–MS analysis appeared to be a good screening-confirmation test scheme for the analysis of K in urine. Twenty of the 22 positive urine specimens contained all three analytes simultaneously, with DHNK showing the highest and K the lowest concentrations.

Keywords: Ketamine; Norketamine; Dehydronorketamine; GC–MS; ELISA

1. Introduction

Club drugs are commonly used at rave parties, nightclubs, and music festivals to enhance sensory stimulation and social intimacy. They are inexpensive and easily distributed as small pills or in powder or liquid forms that are taken orally and often in combination with alcohol, or other drugs, to enhance their effect. Club drugs are especially popular among youngsters for recreation purpose.

Ketamine (K), one of the most widely used club drugs and a parenterally administered anesthetic agent, exhibits sedative, amnestic, and analgesic properties [1]. Abused at a higher dose than normally used for anesthetic purpose, ketamine generates effects similar to those produced by phencyclidine (PCP) with visual effects from lysergide (LSD) use. The over all self-administration behavior is similar to that exhibited by central nervous system depressant drugs in animal studies [2].

In recent years, the abuse of K has dramatically increased worldwide [3–5] requiring the development of effective screening and confirmation methods. Since 1970s, several analytical methods for the determination of K and its metabolites in human urine, plasma, and hair have been developed. These methods were based on gas chromatography with flame-ionization detector (GC-FID) [6], nitrogen–phosphorus detector (GC-NPD) [7], and high-performance liquid chromatography (HPLC) [8]. More specific methods using mass spectrometer as the detector, i.e. gas chromatography–mass spectrometry (GC–MS) [6,7,9–18] and liquid chromatography–mass spectrometry (LC–MS) methods [18,19], have been reported. More recently, highly specific approaches based on liquid chromatography–tandem mass spectrometry (LC–MS–MS) [20] and headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC–MS) [21] have also been advocated as effective "screening" methods.

While LC–MS approaches and GC–MS-based screening methodology may serve specific needs, two-step testing strategy, with each based on a different underlying principle, is

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currently considered the most effective approach under high-volume testing environment. With this in mind, this study has established a correlation of test data between the preliminary and the confirmatory test methods. Furthermore, the confirmatory GC–MS method established in this study achieved better sensitivity and reduced analytical time and cost by eliminating the derivatization step and shortening the chromatographic run time.

2. Experimental

2.1. Chemicals and reagents

K, ketamine-d_4 (K-d_4), norketamine (NK), norketamine-d_4 (NK-d_4) were purchased from Cerilliant (Austin, TX, USA). Dehydronorketamine (DHNK, >99.5% purity) was synthesized by the Department of Chemistry, National Chung-Hsing University (Taichung, Taiwan, ROC). HPLC-grade methanol was purchased from Mallinckrodt (Phillipsburg, NJ, USA). Ethyl acetate, ammonium hydroxide, sodium bicarbonate, and sodium carbonate were all reagent grade. Ultra-pure water was produced with a Milli-Q purification system from Millipore (Bedford, MA, USA). Oasis HLB solid-phase extraction (SPE) cartridges (3 CC, 60 mg) were purchased from Waters Corp. (Milford, Ma, USA). Ketamine ELISA kits were purchased from Neogen Corporation (Lexington, KY, USA).

Standard stock solutions of K, NK, and DHNK (100 μg/mL) were prepared in ethanol and stored at −20 °C. Working standard solutions (K, NK, DHNK) and internal standard solutions (K-d_4, NK-d_4) for calibration and quality control were prepared in ethanol (10 μg/mL in each) and stored at 4 °C. Sodium carbonate–sodium bicarbonate buffer (carbonate-bicarbonate buffer, pH 9.5) was prepared by dissolving 2 g sodium carbonate and 9 g sodium bicarbonate in 1 L ultra-pure water and kept at room temperature. The rinse solution (50:50:2, methanol/water/ammonium hydroxide) for SPE was prepared daily.

2.2. Test specimens, calibrators, controls, and sample preparation

Two hundred and six urine specimens were systematically sampled nationwide from suspected drug users submitted by law enforcement agencies during the summer of 2004.

The six calibrators contained 30, 60, 100, 400, 700, and 1000 ng/mL of K, NK, and DHNK, respectively, and 100 ng/mL of K-d_4 and NK-d_4. Quality controls contained 40, 200, and 900 ng/mL of the analytes as the low, medium, and high controls, respectively. They were prepared by separately adding designated amounts of the working standard solution into 1-mL drug-free urine.

K-d_4 and NK-d_4 were used as the internal standards for the quantitation of K and NK, respectively. NK-d_4 was also used as the internal standard for DHNK for lack of deuterated DHNK. Test specimens, calibrators, and controls were first spiked with 10-μL internal standard solution (equivalent to 100 ng/mL internal standards) and then alkalized with 1-mL carbonate–bicarbonate buffer and vortexed. Extraction was proceeded with Oasis HLB SPE cartridges on a Zymark automated solid-phase extraction system (Hopkinton, MA, USA). The cartridge was first conditioned with 2-mL methanol, followed by 2-mL ultra-pure water and then 2-mL carbonate–bicarbonate buffer. After loading the sample, the cartridge was washed with 2-mL rinse solution. Analytes retained were subsequently eluted with 2-mL methanol following a 0.1-min nitrogen purge. The methanol eluate was evaporated to dryness at 40 °C under a nitrogen stream and reconstituted with 100-μL ethyl acetate prior to GC–MS analysis.

2.3. GC–MS confirmation

An Agilent 6890 GC/5973 MSD system was adopted in this study using a HP-5MS column (30 m × 0.25 mm I.D., 0.25 μm film thickness). GC oven temperature was programmed to rise

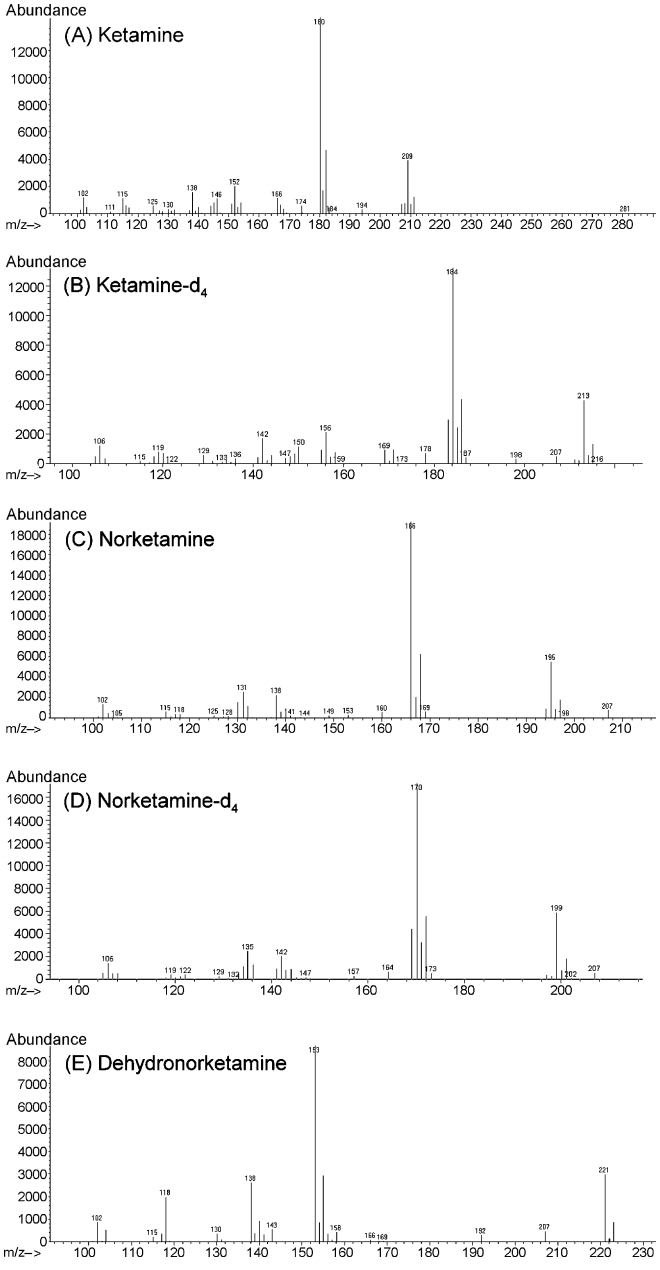

Fig. 1. Full scan mass spectra of K, K-d_4, NK, NK-d_4, and DHNK.