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Determination of ketamine, norketamine and dehydronorketamine in urine by hollow-fiber liquid-phase microextraction using an essential oil as supported liquid membrane☆

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ABSTRACT

Here, we present a method for the determination of ketamine (KT) and its main metabolites, norketamine (NK) and dehydronorketamine (DHNK) in urine samples by using hollow-fiber liquid-phase microextraction (HF–LPME) in the three-phase mode. The fiber pores were filled with eucalyptus essential oil and a solution of 1.0 mol/L of HCl was introduced into the lumen of the fiber (acceptor phase). The fiber was submersed in the alkalinized urine containing 10% NaCl, and the system was submitted to lateral shaking (2400 rpm) during 30 min. Acceptor phase was withdrawn from the fiber, dried and the residue was then derivatized with trifluoroacetic anhydride (TFAA) for further determination by gas chromatography–mass spectrometry (GC–MS). The calibration curves were linear over the specified range and limits of detection (LoDs) obtained for KT, NK and DHNK were below the cut-off value (1.0 ng/mL) recommended by the United Nations Office on Drugs and Crime (UNODC). A totally "green chemistry" approach of the sample extraction was obtained by using essential oil as a supported liquid membrane in HF–LPME. The developed method was successfully validated and applied to urine samples collected from two clinical cases in which KT was suspected to be involved.

1. Introduction

Ketamine (KT) is a *N*-methyl-D-aspartate (NMDA) receptor antagonist used as anesthesic in both animals and humans [1]. It was initially abused by medical personnel due to its hallucinogenic properties, and gradually became popular among young user population at dance and rave parties, being one of the recreational drugs known as "club drugs" [2,3]. KT is an odorless, tasteless and colorless drug and it can be added to beverages, without being perceived by the victim, promoting stupor and sedation together with amnesia. Because of its pharmacological properties, this drug is also misused by offenders in cases of drug-facilitated crimes (DFC) [4–6].

After administration of KT in humans, this substance is broken down into norketamine (NK), an active metabolite. NK is then dehydrogenated generating dehydronorketamine (DHNK). KT and its main metabolites (NK and DHNK) are further transformed by hydroxylation and conjugation prior to elimination (Fig. 1). Approximately 2% is excreted as parent drug, 2% as NK, 16% as DHNK and the rest as conjugates of hydroxylated metabolites [4,7]. According to the Society of Forensic Toxicologists (SOFT) and United Nations Office on Drugs and Crime (UNODC), KT and NK are the target analytes in toxicological analysis with suspected involvement of this drug using urine as biological matrix [6,8]. In spite of the absence of DHNK in the SOFT and UNODC list of targeted analytes, this metabolite has also been indicated as a biomarker of administration of KT in the scientific literature [4,9–11]. In fact, following the administration of a single oral dose of KT (50 mg) in six volunteers, KT and NK were detected in urine up to 5 and 6 days, respectively, and DHNK could be detected for up to 10 days [12].

Urine samples continue to be widely used as a biological matrix for the analysis of psychoactive substances in forensic cases because of the large sample volume that can be collected for

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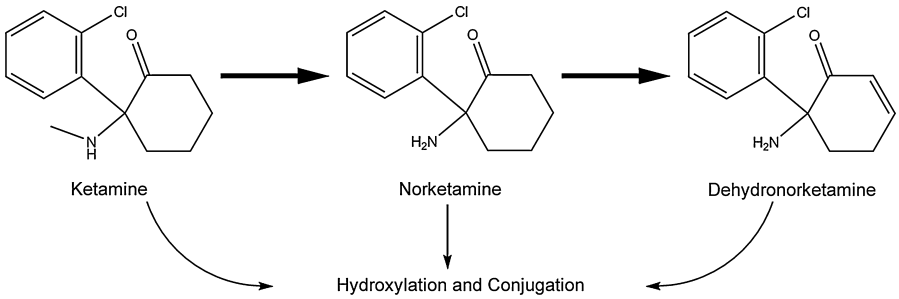

Fig. 1. Chemical structure of target drug analytes.

analysis, the relative simplicity of the sample preparation and a wider drug detection window compared to blood [6,13].

Some analytical methods have been described in the scientific literature for the determination of KT and its main metabolites alone or in combination with other psychoactive substances in urine samples. Gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS) are the main techniques used in the majority of these methods. Sample preparation techniques used in these chromatographic methods include the conventional liquid–liquid extraction (LLE) [3,7,10], solid-phase extraction (SPE) [4,9,11–14] and the use of polyvinylidene difluoride (PVDF) filter syringes [15]. Miniaturized techniques, such as solid-phase microextraction (SPME) and liquid-phase microextraction (LPME) have been scarcely employed for this purpose [2,16].

Over the last decade, the development of miniaturized extraction procedures, especially LPME, has become an encouraging field in analytical chemistry. In hollow fiber–liquid phase microextraction (HF–LPME), a water immiscible solvent is immobilized as a thin supported liquid membrane (SLM) into the pores of a porous hollow fiber. The lumen of the fiber is filled with acceptor solution and the system is placed in contact with the sample (donor phase). The analytes are extracted from the sample (aqueous), through the SLM (organic) and finally into the acceptor solution. The acceptor solution can be an organic solvent, providing a two-phase extraction system or it can be an aqueous solution, providing a three-phase extraction system. In the three-phase extraction system, analytes must be ionized, and consequently, trapped in the lumen of the hollow fiber. Due to the high sample-to-acceptor volume ratio, very high analyte enrichments can be obtained by the use of HF–LPME, especially in the three-phase mode. Also, excellent clean-up has been reported from complex biological matrices because the pores size of the fiber provides microfiltration of macromolecules [17,18].

In the three-phase mode, besides obtaining a better recovery compared to two-phase, this technique allows easy drying and subsequent derivatization of the analytes, a chemical reaction that increases the selectivity and stability of the compounds. Therefore, an interesting perspective of three-phase mode is the possibility of performing a totally "green analytical chemistry" by the use of fatty oils or essential oils as SLM instead of using organic solvents. This type of extraction can eliminate the use of hazardous organic solvents in sample preparation methods based on HF–LPME [18,19].

The aim of the present study was to develop a method for the determination of KT and its main metabolites (NK and DHNK) in urine samples using hollow-fiber liquid phase microextraction (HF–LPME) in three-phase mode and gas chromatography–mass spectrometry (GC–MS). The method was fully validated and successfully applied to urine samples collected from two clinical cases, confirming the suspicion of ketamine exposure in these cases.

2. Experimental

2.1. Reagents and reference standards

Ketamine (KT), norketamine (NK) and dehydronorketamine (DHNK) solutions (1.0 mg/mL) in methanol and the internal standards (IS) ketamine-d4 (KT-d4) and norketamine-d4 (NK-d4) solutions (1.0 mg/mL) also in methanol were purchased from Cerilliant Analytical Reference Standards® (Round Rock, TX, USA). Dihexyl ether, 1-nonanol, undecane, decanol, 1-octanol, xilol, trifluoroacetic anhydride (TFAA) and ethyl acetate were purchased from Sigma-Aldrich® (MO, USA), while sodium hydroxide and hydrochloric acid were purchased from Merck® (Darmstadt, Germany). Essential oils of canola, clove and peppermint were obtained from Mapric® (São Paulo, SP, Brazil). Essential oil of eucalyptus was purchase from Mapric® (São Paulo, SP, Brazil) and Natural Pharma Ltda® (São Paulo, SP, Brazil), while soybean oil and olive oil from Bunge Brazil® (São Paulo, SP, Brazil) were obtained from a local grocery.

2.2. Preparation of standard solutions

Working solutions of KT, NK, DHNK at concentrations of 1.0 μg/mL and 0.1 μg/mL were prepared with methanol in volumetric glassware. Stock solutions were stored refrigerated (−20 °C) when not in use. Working solutions of the IS (KT-d4 and NK-d4) at a concentration of 1.0 μg/mL were also prepared in methanol.

2.3. Instrumentation

Hollow-fiber Q3/2 Accurel KM polypropylene (600 μm i.d., 200 μm wall thickness and 0.2 μm pore size) was purchased from Membrana (Wuppertal, Germany). Gel-loading pippeter tips Round CC 4853 (0.5 mm; 1–200 μL) were purchased from Costar (Corning, NY, USA). Extractions were conducted using a multi-tube vortexer model VWR VX-2500 (Thorofare, NJ, USA). The analyses were performed using an Agilent 6850 Network GC System gas chromatograph coupled with an Agilent 5975 Series quadrupole mass seletive detector (MSD) (Wilmington, DE, USA). Samples were injected into the GC–MS by means of an autosampler (Agilent 7693). Injections were made in the splitless mode (2 min and afterward split vent was turned on in a ratio of 1:50). Chromatographic separation was achieved on a HP-5MS fused-silica capillary column (30 m × 0.25 mm × 0.1 μm film thickness) using helium as the carrier gas at 1.0 mL/min in a constant flow rate mode. The column oven temperature program was as follows: first held at 100 °C, then programed at 10 °C/min to 200 °C (hold 1 min), then 5 °C/min at 210 °C (hold 1 min); 40 °C/min at 300 °C (hold 1 min). Injection port and transfer line were 260 °C and 280 °C, respectively. The MS was operated by electron ionization (70 eV) in selected ion monitoring (SIM) mode. The following ions were chosen for SIM analyses (quantification ions underlined): KT: