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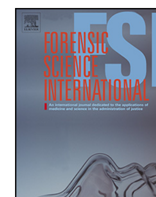

Chiral separation and determination of ketamine and norketamine in hair by capillary electrophoresis

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ABSTRACT

Ketamine, traditionally available as racemic mixture, has recently become available in the form of the single *S*-enantiomer, due to its higher anaesthetic potency associated with faster recovery times. The different pharmaceutical forms and the different pharmacodynamics of the two enantiomers imply the need for a chiral method, since most available analytical methods for biological matrices are not enantioselective.

The method herein showed consists of simple capillary zone electrophoresis (CZE) for the chiral separation of ketamine and its major metabolite, norketamine, in hair specimens. After liquid-liquid extraction, the samples were electrokinetically injected and analysed in CE (running buffer: 15 mM Tris phosphate pH 2.5, containing HS-γ-CDs, 0.1%, w/v). A complete separation of both racemic ketamine and norketamine in the respective enantiomers was obtained in less than 10 minutes. Limit of detection (LOD) and limit of quantification (LOQ) were 0.08 ng/mg and 0.25 ng/mg, respectively. Percent recovery varied from 49% to 91% for all four enantiomers. Matrix effect on spiked hair samples demonstrated values ranging from 63% to 119%. Linearity was estimated using a calibration curve consisting of five concentration levels for each enantiomer (0.5 – 8.0 ng/mg); the regression coefficients (R^2) of weighted ($1/x^2$) linear regression were all >0.988.

The method is suitable for the analysis of real-world hair samples in order to investigate ketamine chronic abuse and to discriminate between the type of abused drug, either single enantiomer or racemic drug.

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1. Introduction

Ketamine, a phencyclidine analogue, is a dissociative anaesthetic that, in addition to analgesia, induces loss of consciousness, amnesia and immobility. First produced as Ketalar® in 1962, it was used to treat American soldiers during the Vietnam war and released for civilian use in 1970 [1]. It has been abused since the 1980s for its stimulant, dissociative and hallucinogenic effects particularly by the young generations, therefore its monitoring is valuable for traffic and workplace safety. It is nowadays still used in paediatric emergency retrieval and in veterinary surgery, because of a reduced tendency to give respiratory depression. In fact, its main advantage is to induce profound analgesia and amnesia, while maintaining the cardiopulmonary functions and the protective airway reflexes stable. The main mechanism of action of ketamine on the central nervous system (CNS) occurs as a non-competitive antagonism on the *N*-methyl-D-aspartate (NMDA) receptor Ca^{2+} channel pore. The NMDA channel block, together with the reduction of presynaptic release of glutamate, appear to be the primary mechanisms of the anaesthetic and analgesic actions of ketamine. There is also evidence that ketamine is capable of interacting with opioid receptors and, specifically with μ and κ. Furthermore it acts as an antagonist in the interaction with monoaminergic, muscarinic and nicotinic receptors [2]. Ketamine undergoes extensive hepatic first-pass metabolism to produce various free and glucorinated hydroxylated derivatives [3]. However, its main metabolic pathway occurs trough *N*-demethylation to norketamine (Fig. 1), which appears to have 20–30% activity of its parent drug.

Starting from the 1980s, ketamine began to be a widespread drug of abuse in many countries and, quite recently, also in Italy. Providing with hallucinogenic effects at sub anaesthetic doses, it is often abused to achieve an out-of-body experience by entering the so-called "*K-hole*". Because of these desired effects, ketamine appears particularly dangerous with regards to traffic and workplace safety.

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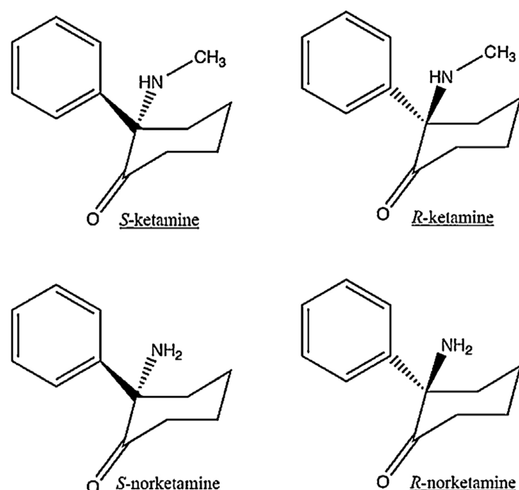

Fig. 1. Structures of the enantiomers of ketamine and norketamine.

Being a chiral drug, ketamine exists in the form of a racemic mixture of its *S*- and *R*- enantiomers. Interestingly, this drug belongs to the infrequent class of chiral compounds for which the sign of rotation changes when the free base is mutated into the hydrochloride salt with preservation of the absolute configuration [4].

The *S*- enantiomer of ketamine recently received peculiar attention since it showed a different pharmacodynamic behaviour, when compared to the *R*-enantiomer and/or to the racemate. It proved a four times higher affinity for the phencyclidine site of the NMDA receptor, as well as a greater potency when compared to *R*-ketamine and to the racemic mixture [2,5]. Since lower doses of the *S*-enantiomer are satisfactory to maintain an equivalent state of anaesthesia, fewer side effects and shorter recovery times are expected with the single enantiomer preparation [6–11]. For these reasons, *S*- ketamine has been released in some countries as an alternative to the racemic ketamine preparations. The different pharmacodynamic behaviour of the two enantiomers implies the need for a chiral analysis method that, to the best of our knowledge, until now, has not been reported in hair samples, particularly including both parent drug and major metabolite. Currently, GC or LC coupled to MS are the techniques of choice for the determination of ketamine, its main metabolite norketamine and, at times, dehydronorketamine in urine and blood samples. The reported LODs (limit of detection) range from 0.02 to 19.00 ng/mL [12–26]. Also, in cases where hair was the biological matrix of interest, GC or LC coupled to MS was the analytical approach of choice with LODs ranging from 2.0 pg/mg to 0.5 ng/mg [27–35]. However, no one of the available analytical methods is enantioselective, showing a clear lack of methodologies.

Because of the availability in the market of both racemic ketamine and its *S*-enantiomer, the chiral analysis herein presented may reveal the type of drug taken by an individual. On the other hand, the chiral analysis of ketamine could also disclose an enantioselective metabolism of the drug.

Consequently, the aim of the present work was to develop an enantioselective CZE method for the separation of ketamine and its major metabolite, norketamine, and to validate its application in hair samples.

2. Materials and methods

2.1. Materials

All used chemicals were of analytical grade. Racemic ketamine HCl and racemic norketamine HCl solutions in methanol (1 mg/mL, 3.66 mM) were purchased from Cerilliant (Round Rock, USA). *S*-and *R*-ketamine, *S*- and *R*-norketamine were kindly provided by the Clinical Pharmacology Laboratory of the University of Bern (Bern, Switzerland). Lamotrigine (used as internal standard, IS) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Tris was purchased from Amersham Biosciences (Uppsala, Sweden) and H_3PO_4 (85%) was from Sigma Aldrich (Steinheim, Germany). HCl 37%, NaOH and *n*-Hexane were from Carlo Erba Reagents (Milano, Italy). Ethyl acetate was obtained from VWR International S.A.S. (Fontenay-sous-Bois, France). Highly Sulfated-γ-Cyclodextrin (HS-γ-CDs) 20% (w/v) aqueous solution (Lot M211145) was kindly donated from Beckman Coulter Inc. (Brea, CA, USA). Milli-Q water produced from Millipore Ultra-Pure Water system (Millipore, Bedford, MA, USA) was used for preparation of the buffers and all aqueous solutions.

2.2. CE system and software

All CE experiments were performed using a P/ACE MDQ electrophoresis apparatus equipped with UV-DAD detection (Beckman Coulter Inc., Brea, CA, USA). The wavelength of detection was set at 200 nm. An uncoated fused-silica capillary of 50 μm i.d. and 45 cm total length (35 cm to the detection window) was utilized. The capillary was washed each day of use before analysis runs with 1 M NaOH, 0.1 M NaOH, water, running buffer at 20 psi for 5 min for each solution. Prior to each run, the capillary was rinsed with water, followed by running buffer at 20 psi for 1 min each. The capillary cartridge temperature was maintained at 20 °C. Separation occurred at a voltage of 20 kV, in 15 mM Tris phosphate (pH 2.5) containing 0.1% HS-γ-CDs (w/v). Identification criteria were based on the relative migration times of the analytes/internal standard. Data were collected and analysed by using MDQ 32 Karat software from Beckman Coulter.

2.3. Preparation of standard solutions

Ketamine and norketamine stock solutions were prepared in methanol at a concentration of 0.5 mg/mL (1.83 mM) of each enantiomer; the working standard solutions were prepared in water at concentrations ranging from 5 μg/mL to 1 ng/mL from the stock solutions. Lamotrigine (IS) stock solution was prepared in methanol at a concentration of 1 mg/mL; the working standard solutions were prepared in water at concentrations ranging from 5 μg/mL to 100 ng/mL. All solutions were stored at −20 °C until analysis.

2.4. Specimens

Drug-fortified hair samples were obtained by spiking blank hair (collected from eight healthy volunteers that had never used either the target drug nor any other illicit drugs) with appropriate standard solutions. Real-world hair samples ($n = 12$) were also analysed. These samples had been previously collected from subjects whose driving license had been confiscated for drug abuse and who were found “positive” for ketamine at a hair toxicological screening test using a validated UHPLC-QqQ MS non-chiral method (ketamine concentrations ranging from 330 pg/mg to 107 ng/mg).

2.5. Sample preparation procedure

Hair samples were first washed with different solvents as follows: 1% SDS for 15 min, water for 5 min, and finally with methanol for 5 min. Hair was allowed to air dry at room temperature under a fume hood prior to the final washing step with methanol. The purpose of washing hair samples was to remove any potential external contaminant present on the surface.

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