DETERMINATION OF KETAMINE, NORKETAMINE, HYDROXYNORKETAMINE AND DEHYDRONORKETAMINE BY LC-MS/MS IN PLASMA SAMPLES

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ABSTRACT

INTRODUCTION: Ketamine (KT) is a medication used in veterinary, pediatric, and ophthalmological medicine primarily as anesthetic. In recent years, it has also been used recreationally and occasionally as a crime-facilitating drug. Additionally, KT exhibits effects similar to classical antidepressant drugs, making it a consideration in depression treatment. **OBJECTIVE**: To optimize and validate a liquid-liquid extraction (LLE) technique and a bioanalytical method based to quantify ketamine (KT) and some metabolites (norketamine - NK, hydroxynorketamine - HNK, and dehydronorketamine - DHNK) in human plasma samples using LC-MS/MS system. **MATERIAL AND METHODS**: Optimization and validation of bioanalytical method was based on GAC principles. Chromatographic system: SCIEX LC-MS/MS, QTRAP® 5400 model with Analyst® 1.7 (Sciex Singapore) software. Analytes separation: ACE C-8 column (150 x 4.6 mm, 5 µm) at 35 ºC with mobile phase (MP) 10 mM ammonium formate with 0.01% ammonium hydroxide (pH 8.0): acetonitrile (65:35, v/v), flow rate 1 mL/min in isocratic mode and 13 min run time. Calibration curves range: 1 - 200 ng/mLwith IS norketamine-d4. Reference ions were obtained from literature. Extraction procedure: plasma sample (200 µL) was added by NaOH (100 µL) and 1250 µL of ethyl acetate, vortexed and centrifuged. Supernatant was transferred to an Eppendorf, dissolved with MP, evaporated at 45 ºC and centrifuged at 14.000 rpm/8 ºC. An supernatant aliquot of 150 µL was taken to a vial and 50 µL was injected into HPLC. Confidence limits based on RDC ANVISA 27/2012. **RESULTS AND CONCLUSIONS**: Recovery for KT, NK and HNK was close to 100%; for DHNK, 49%. The technique showed linearity (1 – 200 ng/mL), intraday and interday precisions, accuracy and stability (short-term, freeze/thaw, post-processing and long-term) within the limits. Additionally the LC-MS/MS technique proved to be very selective, especially for metabolites; matrix effect and residual below the limits. That technique was applied to two real cases of poisoning and it was identified all analytes in the plasma samples. In conclusion the technique was validated using low plasma volume and proved to be sensitive, selective and efficient for toxicological analyses and bioavailability studies.

Keywords: *Analytical toxicology; drug of abuse; liquid-liquid extraction; liquid chromatography; mass spectrometry.*

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