Quantification of Drugs for Drug-Facilitated Crimes in Human Urine by Liquid Chromatography Tandem Mass Spectrometry

Claudio De Nardi[1], Anna Morando[2], Anna Del Plato[2]

[1]Thermo Fisher Scientific GMBH, Dreieich, Germany

[2]Ospedale "La Colletta", Arenzano, Italy

An analytical method based on liquid chromatography tandem mass spectrometry for the quantification of drugs for drug-facilitated crimes in human urine is described. The method includes ketamine, its metabolites norketamine and dehydronorketamine, phencyclidine and γ-butyrolactone (GBL); the method is also suitable to detect γ-hydroxybutyric acid (GHB) at physiological levels. Sample preparation was based on extraction using three volumes of methanol containing 0.1% formic acid. Mass spectrometric detection was performed by single reaction monitoring (SRM) on a Thermo Scientific™ TSQ Quantum Access MAX™ triple quadrupole using heated electrospray ionization. Linearity of the method was evaluated on duplicate curves for each analyte.

Background: a considerable increase in the number of reported drug-facilitated crimes (DFC) has occurred in recent years. Drugs that can induce a state of semi- or unconsciousness in the victims are most typically used in these cases; victims are usually unable to fight off their attackers and report their inability to prevent the crime as it occurs. Sedative-hypnotic drugs like norketamine, phencyclidine (PCP) and γ-hydroxybutyric acid (GHB) are among the compounds most frequently involved in this kind of offences.

Methods: a calibration curve covering the concentration range 5-200 ng/mL was prepared by spiking blank human urine with methanolic solutions (50x) containing ketamine and its metabolites norketamine and dehydronorketamine and PCP. 300 µL of a 100 ng/mL solution containing ketamine-D4 and PCP-D5 in methanol with 0.1% formic acid were added to 100 µL of each calibrator; a blank urine sample was also added. Due to the high concentrations involved, a calibration curve of GBL in methanol was prepared to cover the concentration range of 1-100 µg/mL; this range was based on the assumption [1,2] of an endogenous level for GHB in urine of 10-50 µg/mL. 100 µL of each calibrator, including a blank, were added to 300 µL of a 50 µg/mL solution of GHB-D6 and GBL-D6 in water/methanol/formic acid 33/67/0.1 (v/vv). All samples from the two extracted calibration curves were vortex-mixed, centrifuged and the supernatant injected onto an LC-MS/MS system. Chromatographic separation was achieved on a Themo Scientific™ Hypersil GOLD™ column (150 x 2.1 mm, 3

μm) at room temperature using gradient elution with a mobile phase consisting of water and methanol both containing 0.1 % formic acid. Detection was performed by single reaction monitoring (SRM) on a TSQ Quantum Access MAX triple quadrupole mass spectrometer using heated electrospray ionization in positive mode.

Results: the assay proved to be linear for all the analytes of interest in the calibration range specified above using a linear interpolation with 1/x weighing. The percentage bias between nominal and back-calculated concentration for the calibrators was between -10.6% and 12.3%; the correlation factor (R^2) was always above 0.998. The method also proved to be able to detect GHB in human urine above the endogenous level; a signal-to-noise ratio above 400 was obtained for GHB in the three human urine samples analysed.

Conclusions: a liquid chromatography tandem mass spectrometry method for the quantification of a panel of drugs for DFC and their metabolites in human urine has been developed on a TSQ Quantum Access MAX. The instrument proved to have the sensitivity and linearity of response suitable to cover the necessary range of concentration for these drugs.

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