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

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Overview

Purpose: To implement a liquid chromatography tandem mass spectrometry method for forensic toxicology for the quantification of drugs for drug-facilitated crimes in human urine on a Thermo Scientific™ TSQ Access MAX™ triple stage mass spectrometer; the method includes ketamine, its metabolites norketamine and dehydronorketamine, phencyclidine and γ-butyrolactone (GBL); the method is also suitable for the detection of γ-hydroxybutyric acid (GHB) at physiological levels.

Methods: Following extraction using three volumes of methanol containing 0.1% formic acid, samples were injected onto a Thermo Scientific™ Accela™ 600 HPLC system connected to a TSQ Access MAX triple stage mass spectrometer using a heated electrospray source. Data acquisition was performed by selected reaction monitoring (SRM) and results were processed using Thermo Scientific™ Xcalibur™ software.

Results: The analytical method proved to be linear for all the analytes of interest in the calibration range 5-200 ng/mL for ketamine, its metabolites and phencyclidine and 1-100 µg/mL for GBL; the percentage bias between nominal and back-calculated concentration for the calibrators was between -10.6% and 12.3% and the correlation factor (R^2) always above 0.998. The method also proved to be able to detect GHB in human urine above the endogenous level.

Introduction

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 and GHB are among the compounds most frequently involved in this kind of offences. We hereby report the implementation of an LC-MS/MS analytical method for the quantification of ketamine and its metabolites norketamine and dehydronorketamine, phencyclidine, GHB and its metabolite GBL in human urine.

Methods

Sample Preparation

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 phencyclidine. 300 µL of a 100 ng/mL solution containing ketamine-D4 and phencyclidine-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.

Nominal concentrations of calibrators are reported in Table 1.

Liquid Chromatography

The following LC conditions were used:

LC system	Thermo Scientific™ Transcend™ I TLX-1 system used in LX mode			
Analytical column	Thermo Scientific™ Hypersil GOLD™ 150x2.1mm (3µ) @ RT			
Mobile phase	A. 0.1% formic acid in water			
	B. 0.1% formic acid in methanol			
Gradient Profile	Time (min)	Flow Rate (mL/min)	A (%)	B (%)
	0.0	0.2	98	2
	0.2	0.2	98	2
	5.0	0.2	90	10
	5.1	0.5	0	100
	7.0	0.5	0	100
	7.1	0.5	98	2
	10.0	0.2	98	2
Injection Volume	10µL			

TABLE 1. Concentrations of calibrators

Analyte	Units	CAL 1	CAL 2	CAL 3	CAL 4	CAL 5	CAL 6	CAL 7
Ketamine	ng/mL	5	10	20	50	100	200	N/A
Norketamine								
Dehydro norketamine								
Phencyclidine								
GBL	µg/mL	1	2	5	10	20	50	100

Mass Spectrometry

The LC system was connected to a TSQ Access MAX triple-stage quadrupole mass spectrometer; acquisition time ranges were used for each analyte and the following MS conditions were used:

Source type	Heated electrospray ionization (HESI) in positive mode
Vaporizer temp	325°C
Capillary temp	280°C
Spray voltage	4000 V
Sheath gas	35 AU
Sweep gas	0 AU
Auxiliary gas	10 AU
Data acquisition mode	Selected reaction monitoring (SRM)
Chrom filter peak width	5.0 s
Collision gas pressure	1.5 mTorr
Cycle time	0.400 s
Q1 (FWMH)	0.7
Q3 (FWMH)	0.7
SRM transitions	See Table 2

TABLE 2. SRM transitions

Analyte	Start Time (min)	Stop Time (min)	Tube Lens (V)	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)
Ketamine	6.0	8.0	70	238.0	125.1	28
Norketamine	6.0	8.0	70	224.0	125.1	24
Dehydro norketamine	6.0	8.0	70	222.0	142.1	20
Ketamine-D4	6.0	8.0	70	242.0	129.1	26
Phenylcyclidine	6.0	8.0	40	244.1	86.3	12
					91.2	28
					159.1	11
Phenylcyclidine-D5	6.0	8.0	60	249.1	86.3	12
					96.2	29
					164.1	11
γ-Hydroxybutyric acid	1.0	5.0	25	105.1	45.6	17
					69.4	10
					87.3	5
γ-Hydroxybutyric acid-D6	1.0	5.0	45	111.1	93.3	5
γ-Butyrolactone	3.0	7.0	60	87.1	43.6	10
					45.6	15
γ-Butyrolactone-D6	3.0	7.0	60	93.1	49.6	15

Data Analysis

Data were quantitated using a linear regression and 1/x weighing was used to build the calibration curves. A maximum percentage bias between nominal and calculated concentration of 15% was set as acceptance criterion for all calibrators.

Results

The assay proved to be linear for all the analytes of interest in the evaluated concentration ranges and correlation factors (R^2) were always above 0.998. The percentage bias between nominal and back-calculated concentration for the calibrators was between -10.6% and 12.3%. A summary of calibration range, intercept, slope and correlation factor for each analyte is reported in Table 3.

A representative calibration curve for GBL is reported in Figure 1. Representative chromatograms at the LOQ for each analyte, including the internal standards, are reported in Figure 2.

TABLE 3. Concentration range, intercept, slope and correlation factor (R^2)

Analyte	Calibration Range	Intercept	Slope	R^2
Ketamine	5 – 200 ng/mL	-0.002	0.004	0.999
Norketamine	5 – 200 ng/mL	0.000	0.003	0.998
Dehydronorketamine	5 – 200 ng/mL	-0.001	0.002	0.999
Phencyclidine	5 – 200 ng/mL	0.000	0.003	0.999
GBL	1 – 100 µg/mL	-0.001	0.008	0.999

Figure 1. Calibration curve for GBL

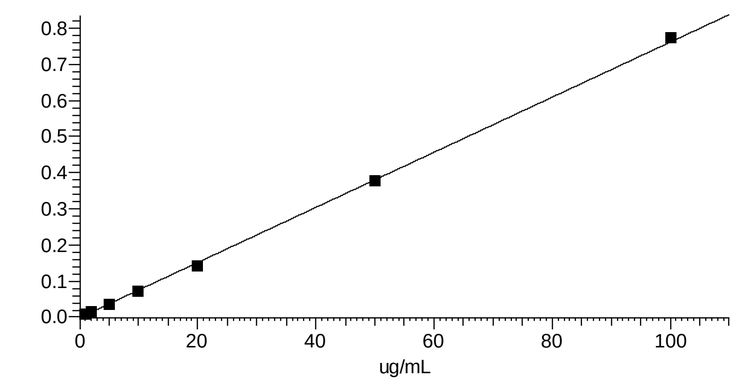
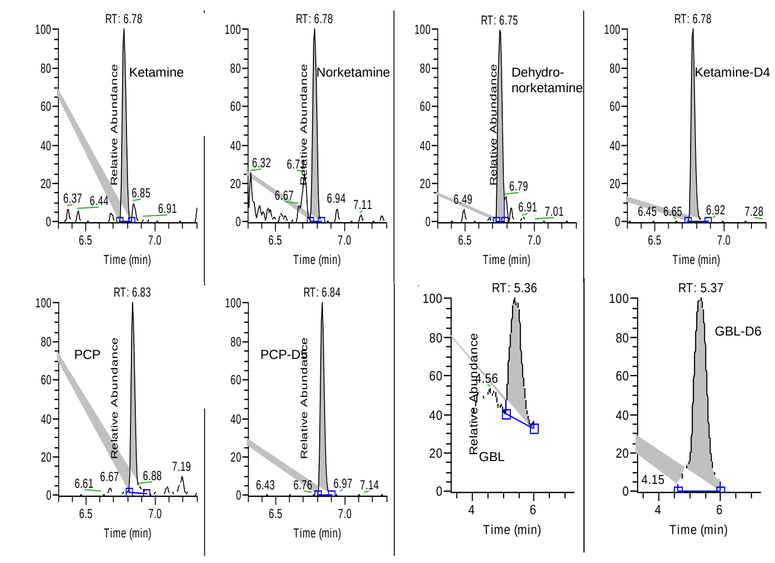
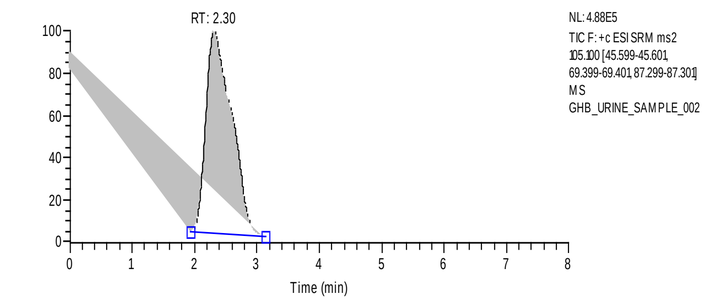


Figure 2. Representative chromatograms of each analyte at the LOQ



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. A representative chromatogram for GHB in a real urine sample is reported in Figure 3.

Figure 3. Representative chromatogram of GHB from a real urine sample



Conclusion

A liquid chromatography tandem mass spectrometry method for forensic toxicology 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.

References

1. Jour An Tox 35 (2011) 8-14
2. Anal Bioanal Chem 406 (2014) 3553–3577

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