

Liquid chromatography-multiple mass spectrometry determination of atracurium and laudanosine in a case of suspected multi-homicide

DONATA FAVRETTO, MARIELA IVANOVA, ALESSANDRO NALESSO,
FRANCA CASTAGNA and SANTO DAVIDE FERRARA

Forensic Toxicology and Antidoping, University Hospital of Padova, Via Falloppio 50, I-35121 Padova, Italy

AIMS: The forensic toxicology literature lack data concerning poisonings by atracurium and only a fatal case, resulting from self-administration, was reported. Atracurium is a non-polarizing skeletal muscle relaxant, used to facilitate endotracheal intubation and to provide skeletal muscle relaxation during surgery or mechanical ventilation. Atracurium is available as 1% solution of the besylate salt, in ampoules of 2.5, 5 or 25 ml, for intravenous administration; controlled ventilation is always necessary. Under physiological conditions (temperature and pH), atracurium is rapidly decomposed by non-specific esterases and by non-enzymatic hydrolysis to give laudanosine and pentamethylene-1,5 diacrylate. The drug is excreted in urine and bile and its elimination half-life is about 20 min. In a case of suspected multi-homicide in a hospital setting, systematic toxicological analyses (STA) were performed in fluids and tissues from six exhumed cadavers; among the various xenobiotics which were identified by STA, laudanosine was detected in specimens from every cadaver. Thus, the possibility of fatal atracurium injection(s) needed to be investigated. A method was developed to quantitate atracurium in body fluids and tissues by liquid chromatography-multiple mass spectrometry (LC-MS/MS). The method is more specific than previous LC-MS methods based on single ion monitoring, allows the simultaneous quantification of atracurium and laudanosine in whole blood and in post-mortem tissues such as heart, lung and liver, and proves to be more suitable than LC-MS/MS methods validated for blood, plasma and serum for forensic toxicological investigations.

METHODS: After laudanosine was detected in the fluids and tissues of the exhumed cadavers by an STA procedure based on GC-MS, a method was developed for the simultaneous quantification of atracurium and its metabolite laudanosine. The method is based on liquid/liquid extraction and LC-MS/MS in positive ion electrospray ionisation conditions, using verapamil as the internal standard. MS/MS experiments were achieved by collisional induced dissociation on an ion trap. Liquid chromatography was performed by gradient elution on a cyanopropyl column. Matrix effects and ion suppression effects were investigated. The method was validated in terms of selectivity, linearity, limits of quantification (LOQ) and of detection (LOD), accuracy, and precision.

RESULTS AND CONCLUSIONS: The method proved to be selective and sensitive, allowing the determination of both analytes at low concentration levels in all matrices. LOQ (the concentration below which the RE was > 20% and the RSD was > 20%) was 1 ng/mL for both laudanosine and atracurium in blood. LOD (the concentration with signal-to-noise ratio of 3) was 0.5 ng/mL for both analytes in blood. Linearity was found between 1 and 1000 ng/mL in all matrices, with correlation coefficients higher than 0.999. Intra-assay precision (as relative standard deviation, RSD, n = 4) at three concentration levels was always better than 16 %, while inter-assay precision (RSD, n = 12) was always better than 20 %. Accuracy (as % bias, i.e. the percentage deviation of the mean from the value of a fortified sample) was determined on the basis of the total data set (n = 12) and was always better than 9%. Toxicological analysis revealed the presence of atracurium and laudanosine in blood, heart, lung and liver sample for all cadavers. Concentration of atracurium in blood was in the range 1,5 - 67,1 ng/mL while laudanosine was quantified in the range 205 - 1466 ng/mL. Other drugs were detected and quantified (among which propofol, morphine, digoxin, amiodarone, tramadol, dobutamine, atropine, clothiapine, phenytoin, ticlopidine, midazolam, paroxetine) either by gas chromatography mass spectrometry (GC-MS) or LC-MS/MS, after solid-phase extraction or liquid/extraction. The toxicology results were evaluated and matched up to case history, clinical evidences and autopsy findings.

KEYWORDS: *Multi-homicide, Atracurium, Laudanosine, HPLC-MS/MS, Post-mortem tissues*

Corresponding author: donata.favretto@sanita.padova.it