Detection and validated quantification of the herbal phenalkylamines and methcatinone in human blood plasma by LC-MS-MS

J. BEYER[1], F. T. PETERS[1], T. KRAEMER[2] and H. H. MAURER[1]

1 Department of Experimental and Clinical Toxicology;

2 Institute of Legal Medicine, Saarland University, D-66421 Homburg (Saar), Germany

AIMS: The herbal stimulants *Ephedra spec*., *Catha edulis*, and *Lophophora williamsii* have been abused for a long time. In recent years, the herbal drug market has grown considerably due to a well-organized publicity in the internet. A number of intoxications involving these herbal stimulants have been reported. Some of the active ingredients of these plants are also ingredients of cold remedies. Therefore, and in order to differentiate the intake of such a cold medication from herbal drug or methcathinone abuse, methods for the determination of these alkaloids and methcathinone, especially in blood plasma, are needed due to the growing importance of this matrix in analytical toxicology.

METHODS: After mixed-mode solid-phase extraction (HCX) of 1 mL of plasma, the analytes ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine, methylephedrine, methylpseudoephedrine, cathinone, mescaline, synephrine, and methcathinone were separated using a Shimadzu Prominence HPLC system with an SCX separation column (Zorbax 300SCX, 2.1 × 150 mm), gradient elution with a mobile phase of 5 mM ammonium formate buffer pH 3/acetonitrile and a total flow of 1.5 mL/min. They were detected using an Applied Biosystems 3200 Q-Trap LC-MS-MS system (ESI, MRM mode). Calibration curves were used for quantification using norephedrine-d3, ephedrine-d3 and mescaline-d9 as internal standards. The method was fully validated according to international guidelines.

RESULTS: The assay was found to be selective for the tested compounds. It was linear from 10 to 1000 µg/L for all analytes. The recoveries were generally larger than 70%. Accuracy, repeatability and intermediate precision were within the required limits. They ranged from -0.8 to 20.0 % for accuracy, from 2.5 to 12.3 % for repeatability, and from 4.6 to 20.0 % for intermediate precision. The limit of quantification was 10 µg/L for all analytes. No instability was observed after repeated freezing and thawing or in processed samples. The applicability of the assay was proven by analysis of authentic plasma samples after ingestion of different cold medications containing ephedrine or pseudoephedrine, and after ingestion of an aqueous extract of *Herba Ephedra*. After ingestion of the cold medications only the corresponding alkaloid was detectable, whereas after ingestion of the herb extract, all six ephedrines contained in the plant were detectable in human plasma.

CONCLUSIONS: The presented LC-MS-MS assay has proven to be applicable for determination of the studied analytes in plasma and for differentiation of an intake of cold medications from herbal drug or methcathinone abuse.

KEYWORDS: *LC-MS-MS; Plasma; Herbal drugs; Phenylalkylamines*

Corresponding author: jochen.beyer@uniklinikum-saarland.de