Studies on the metabolism and the detectability of 4-methyl-amphetamine and its isomers 2-methyl-amphetamine, and 3-methyl-amphetamine in rat urine using GC-MS, LC-MS^n, and LC-HR-MS^n

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Abstract

Aims: 4-Methyl-amphetamine (4-MA) and its isomers 2-methyl-amphetamine (2-MA) and 3-methyl-amphetamine (3-MA) are used as so-called research chemicals. 4-MA has been scheduled in Germany in 2012. The aim of this study was to compare these drugs with respect to their metabolites in rat urine, their detectability within our standard urine screening approaches (SUSA) using GC-MS and LC-MS^n and to differentiate these isomers.

Methods: Urine samples were collected over 24 h from male Wistar rats after administration of each of the drugs for toxicologic diagnostic reasons. For the metabolism study (20 mg/kg BW), urine samples were worked-up either by protein precipitation or by enzymatic conjugates cleavage and solid-phase extraction (HCX), the underivatized and/or acetylated extracts were then analyzed by GC-MS (TF ISQ) and LC-HR-MS^n (TF Orbitrap Velos). For SUSA (3 mg/kg BW), urine samples were worked-up by acid hydrolysis, extraction and acetylation (GC-MS) or protein precipitation (LC-MS^n; TF LXQ). For the differentiation of the isomers, the extracts were derivatized by heptafluorobutyrylation and analyzed by GC-MS.

Results and Discussion: According to the identified metabolites, aromatic and aliphatic hydroxylation could be postulated as the main steps for all isomers. In addition, second hydroxylation followed by partial methylation of one hydroxy group was observed for 2-MA. The hydroxy metabolites were partly conjugated. After low dose application, all studied drugs were detectable by SUSA via their metabolites. Only after heptafluorobutyrylation, the isomers (at least the excreted parent drugs) could be differentiated by different GC retention times.

Conclusion: The three isomers of methyl-amphetamine were extensively metabolized so that the hydroxy metabolites beside the parent compounds could be the targets for urinalysis. Assuming similar metabolism in humans, the authors' SUSAs should be suitable to prove an intake of any of the studied drugs in human urine and differentiation of the three isomers was successful with an additional work-up.

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