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\textsuperscript{n}, and LC-HR-MS\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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 USA 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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