Identification and characterization of the new designer benzodiazepines bromazolam, flualprazolam and fluclotizolam

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BACKGROUND & OBJECTIVES
An increasing number of designer benzodiazepines (DBs) – a class of ‘new psychoactive substances’ (NPS) – was reported to the European monitoring centre for drugs and drug abuse (EMCDDA) in the last years. By the end of 2017 a total of twenty DBs have been reported to the EMCDDA. DBs have become a popular alternative for prescription drugs as self-medication or to counteract the effects and side effects of other drugs. Here we present the characterization of three new DBs that emerged on the European drug market in late 2016 and 2017. In addition, first in vitro metabolic studies were performed to identify phase I metabolites suitable as biomarkers for DB screening in human serum and urine samples.

METHODS

Studies of in vitro phase I metabolism using pooled human liver microsomes (pHLM)

RESULTS

Structure identification and characterization

GC-ESI-MS analysis

Flipchart GC-MS spectra

NMR analysis

Identification of in vitro phase I metabolites

Only two phase I metabolites were detected for the investigated compounds in the in vitro-microsomes assay. One compound observed was hydroxylated, resulting in mono-hydroxylated and dihydroxylated metabolites for all three benzodiazepines.

Parent compounds

For bromazolam and flualprazolam two monohydroxylated metabolites were identified, either hydroxylated at C6 of the diazepine core or at the C6 atom of the benzene ring. For fluclotizolam only one peak representing monohydroxylation was observed. The presence of both hydroxylated isomers due to mixing chromatographic separation.

Separation of the two tentatively coeluting hydroxymetabolites was achieved applying another chromatographic gradient, identifying the same two monohydroxylated isomers as for the other two compounds.

CONCLUSIONS

The pHLM assay showed a distinct metabolic profile for the three compounds matching the metabolic pattern found for other triazolo benzodiazepines so far. Most tentatively identified in vivo phase I metabolites (monohydroxylated metabolites) were detected with relatively high abundances, suggesting these compounds as potential long term biomarkers.

Literature


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