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In vitro Phase I and Phase II metabolism of the new designer benzodiazepine cloniprazepam using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry

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RESEARCH HIGHLIGHTS

- Nine in vitro metabolites of cloniprazepam identified with LC-QTOF-MS after incubation with human liver microsomes.
- Five metabolites proposed as potential analytical targets for cloniprazepam consumption.
- Clonazepam appeared to be the most prominent metabolite of cloniprazepam.

Abstract

Designer benzodiazepines have recently emerged as a class of new psychoactive substances. These substances are used in recreational settings and as alternatives to prescription benzodiazepines as self-medication for patients suffering from anxiety or other mental disorders. Due to the limited information available on the metabolic fate of these new substances, it is challenging to reliably detect their usage in bioanalytical (e.g. clinical and forensic) settings. The objective of this study was to investigate the *in vitro* Phase I and Phase II metabolism of the new designer benzodiazepine cloniprazepam and identify potential biomarkers for its detection in human biological fluids. Cloniprazepam was incubated with human liver microsomes and cytosolic fractions to generate both Phase I and II metabolites. The extracts were analysed using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Identification of the metabolites was performed using two complementary workflows, including a suspect screening based on *in silico* predictions and a non-targeted screening. A total of nine metabolites were identified, eight Phase I metabolites and one Phase II metabolite, of which five were specific for cloniprazepam. Clonazepam was the major metabolite of cloniprazepam. Hydroxy-cloniprazepam, dihydroxy-cloniprazepam, 3-keto-cloniprazepam, 7-amino-cloniprazepam, hydroxy-clonazepam, 7-amino-clonazepam and 3-hydroxy-7-amino-clonazepam were formed through oxidation, hydroxylation, and/or reduction of the nitro-group. Glucuronidated hydroxy-cloniprazepam was the only Phase II metabolite detected. Five metabolites were specific for cloniprazepam. This study provided a set of human *in vitro* biotransformation products which can assist specific detection of cloniprazepam consumption in future studies.