CHARACTERIZATION AND *IN-VITRO* PHASE I METABOLITE IDENTIFICATION OF THE DESIGNER BENZODIAZEPINES CLONAZOLAM, DESCHLOROETIZOLAM, AND MECLONAZEPAM

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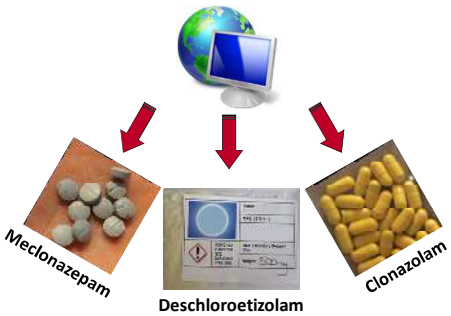
INTRODUCTION

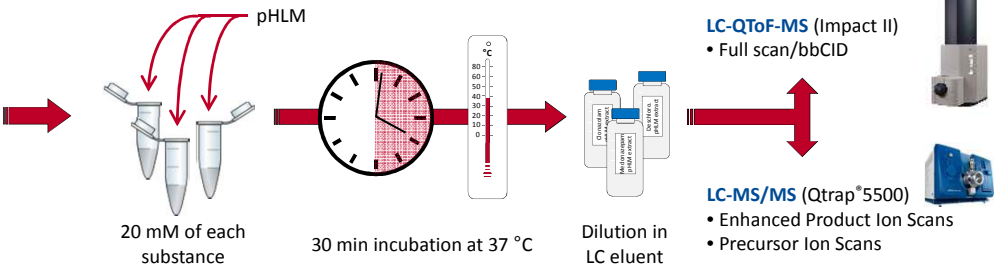
Benzodiazepines are widely prescribed for treatment of seizures as well as sleeping and anxiety disorders but also bear a high risk of misuse and dependency. In 2012, the first designer benzodiazepines were offered in Internet shops as an alternative to prescription-only benzodiazepines. Soon after the first of these compounds were scheduled in different countries, new substances were offered, with clonazolam, deschloroetizolam, and meclonazepam being three of the most recent ones. The present study was set up to characterize these three designer benzodiazepines which recently emerged on the 'legal high' market and to investigate their metabolism *in-vitro* using pooled human liver microsomes (pHLMs). The information gained can be used to update analytical methods for the detection and quantification of benzodiazepines in biological samples.

EXPERIMENTAL

'Online-Shopping'



In-vitro Phase I Metabolite Identification



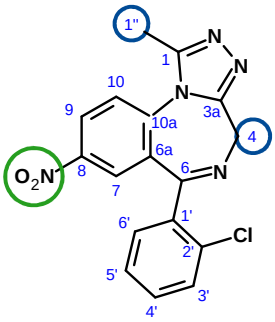
Characterization of Compounds

RESULTS

Characterization confirmed all compounds being those stated. The following metabolites were found in the pHLM-assay by LC-MS/MS (EPI of theoretical metabolite m/z) and confirmed by accurate mass measurements:

○ hydroxylation sites ○ reduction of nitro-group to an amine

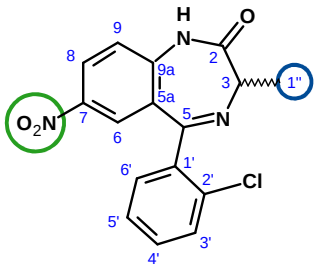
Clonazolam (Clonitrazolam)



1) mono-OH-metabolite
alpha-, or 4-OH-clonazolam

2) reduction of nitro-group
8-amino-clonazolam

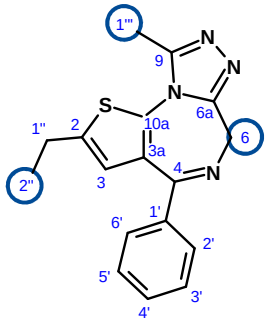
Meclonazepam



1) mono-OH-metabolite
3-OH-methyl-meclonazepam

2) reduction of nitro-group
7-amino-meclonazepam

Deschloroetizolam „Etizolam 2"



1) three mono-OH-metabolites
9-OH-methyl-, 2-OH-ethyl-, 6-OH-deschloroetizolam

2) di-OH-metabolite

The absolute configuration of meclonazepams carbon 3 as well as the exact hydroxylation sites of the mono- and di-OH-metabolites can not be determined sufficiently with the applied MS-techniques. Hence, further investigation using advanced NMR techniques is required. Detailed analytical information can be found in Ref [1].

CONCLUSIONS

The benzodiazepines clonazolam, deschloroetizolam, and meclonazepam were structurally characterized, and their respective *in-vitro* main phase I metabolites identified. Certainly, all described metabolites are prone to undergo further phase II metabolic transformations *in-vivo*, such as O- and N-glucuronidation, and acetylation of the amino moiety of the respective metabolites of clonazolam and meclonazepam. Future studies should include verification of the proposed positions of hydroxylation, comparison of the identified metabolites with metabolites formed *in-vivo*, and assessment of basic pharmacokinetic data.
Clonazolam can be assumed to show a rather high pharmacological potency due to its triazolo-moiety. Consequently, the assumed blood concentrations of this drug can be expected to be relatively low, making it difficult to detect the drug in biological samples. This poses particular challenges for toxicologists analyzing samples of suspected drug-facilitated crime victims. The use of up-to-date MS-based screening and quantitation procedures seems mandatory, because the widely used immunoassays may not be able to detect such low concentrations.

[1] Huppertz et al. (2015) Characterization of the four designer benzodiazepines clonazolam, deschloroetizolam, flubromazolam, and meclonazepam, and identification of their in vitro metabolites. Forensic Toxicol DOI 10.1007/s11419-015-0277-6

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