

# Characterization and *in vitro* phase I microsomal metabolism of designer benzodiazepines - an update comprising adinazolam, cloniprazepam, fonazepam, 3-hydroxyphenazepam, metizolam, and nitrazolam

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## Introduction

Designer benzodiazepines represent the latest class of new psychoactive substances (NPS). While other classes of NPS such as cannabinoid receptors agonists or synthetic cathinones are mainly consumed for hedonistic reasons, designer benzodiazepines may also be consumed for 'self-medication' by persons with anxiety disorders or by users of stimulant and hallucinogenic drugs ('stand-by medication' to counteract unpleasant overstimulation). In the present study, five benzodiazepines and one thienodiazepine offered as research chemicals on the Internet were characterized and their main *in vitro* phase I microsomal metabolites identified. The information obtained can be used to update analytical methods for the detection and identification of benzodiazepines in biological samples.

## Workflow

**1. Product Monitoring**

Research chemicals

- Adinazolam
- Cloniprazepam
- Fonazepam (Norflunitrazepam)
- 3-Hydroxyphenazepam
- Nitrazolam

Tablets

Metizolam

All products were obtained via Internet shops in 2015

**2. Characterization**

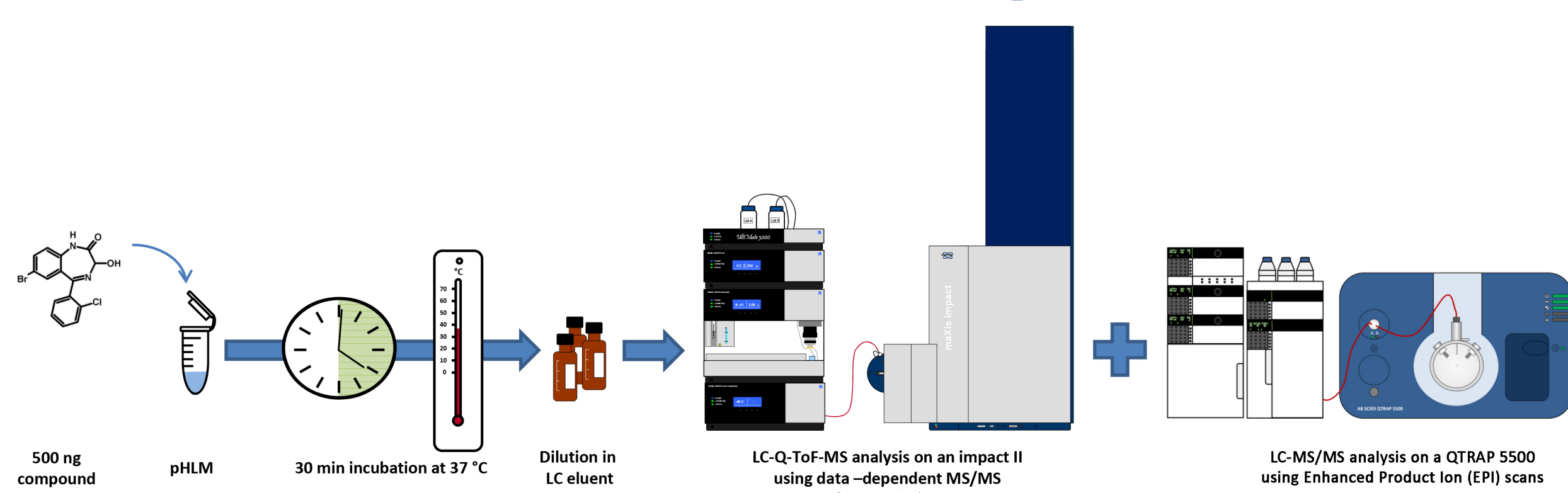
NMR LC-Q-ToF-MS GC-MS LC-MS/MS

The declared structural formula was confirmed by the applied techniques for all compounds.

For adinazolam and fonazepam no NMR analysis was performed, since the compounds are included in the MPW library

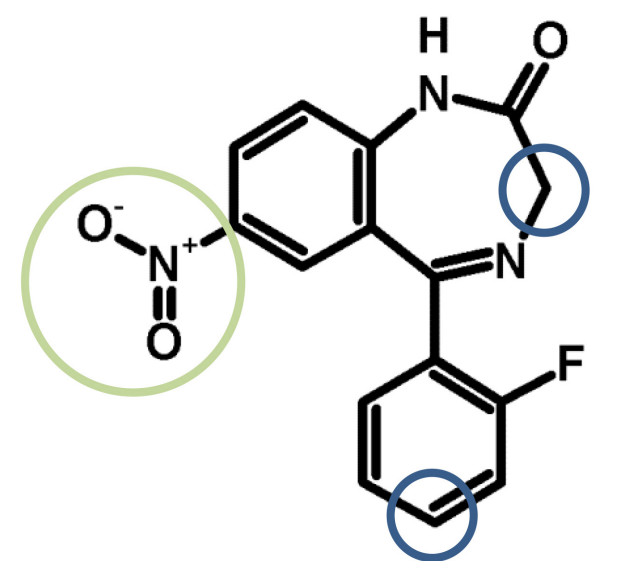
## 3. *In vitro* phase I microsomal metabolism

### Pooled human liver microsomes (pHLM) incubation



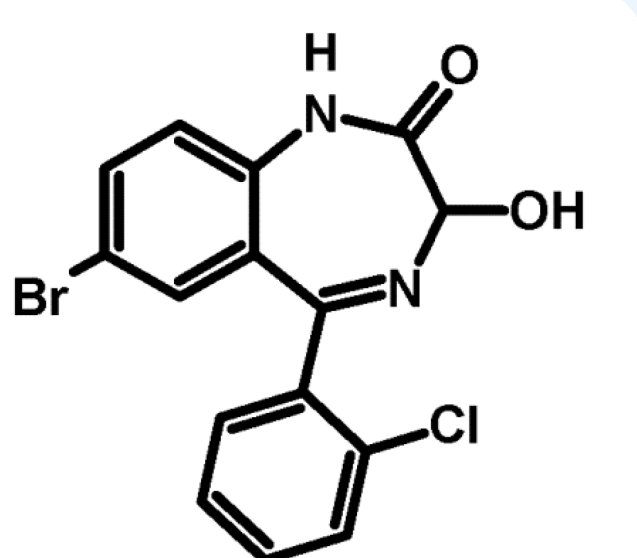
### Fonazepam (Norflunitrazepam)

Signals at  $m/z$  values corresponding to two mono-hydroxylated metabolites and to 7-aminofonazepam (7-aminonorflunitrazepam) could be observed. One of the monohydroxylated compounds could be confirmed as the minor flunitrazepam metabolite 3-hydroxy-norflunitrazepam, which is by itself sold under the name nifoxipam as a 'research chemical' online.

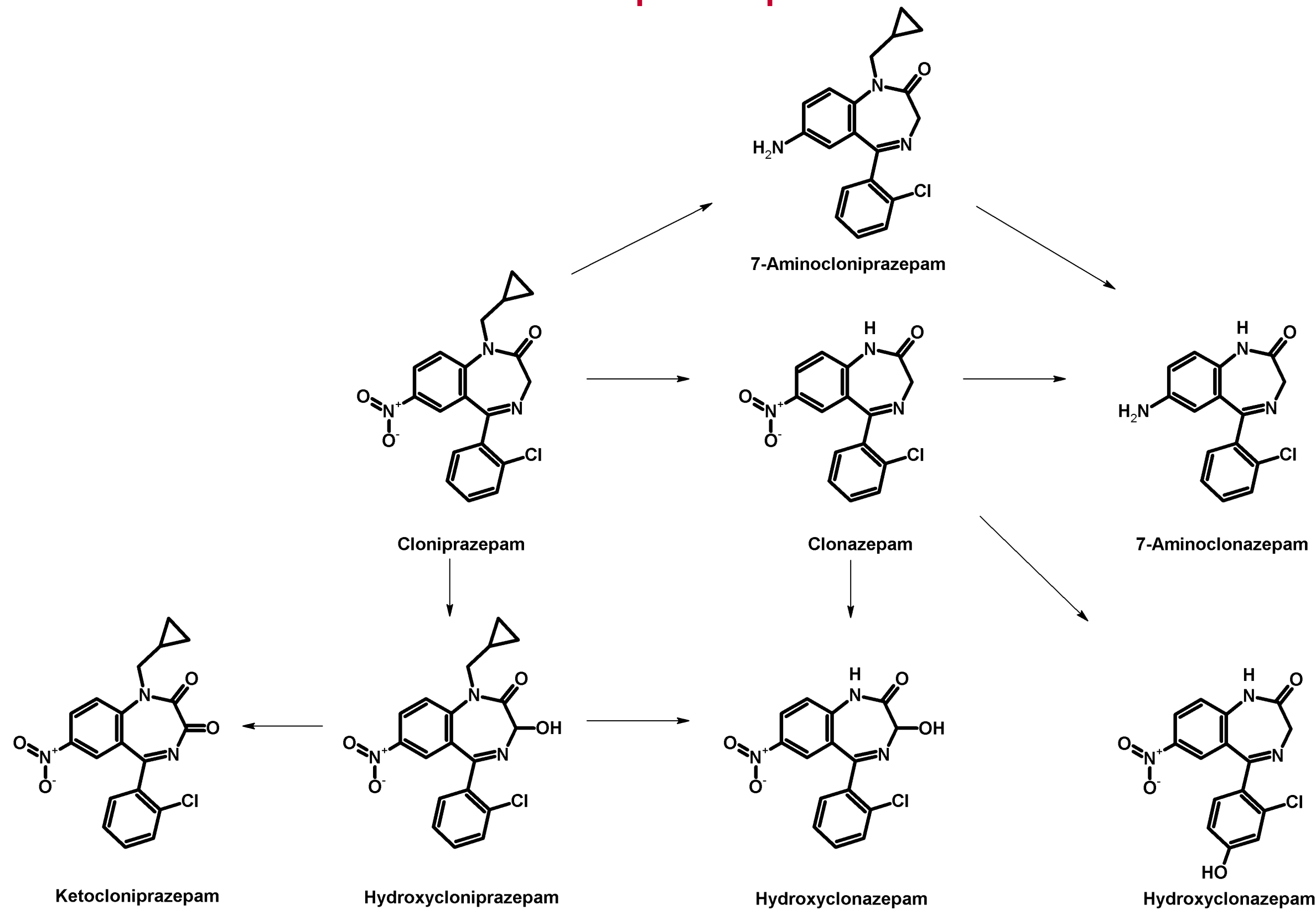


### 3-Hydroxyphenazepam

No phase I metabolite could be detected in the *in vitro* samples using pHLM incubation. This result comes not unexpected as other 3-hydroxylated benzodiazepines, like lorazepam and oxazepam, do not undergo further phase I metabolism to a significant extent.

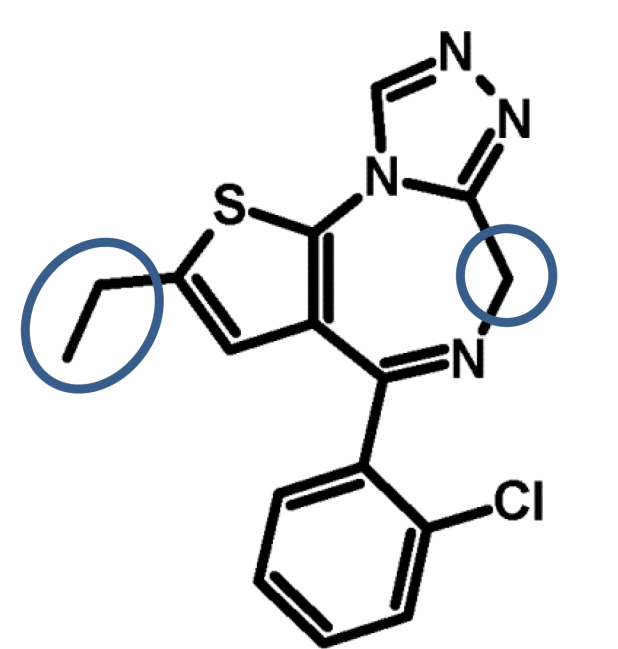


### Cloniprazepam



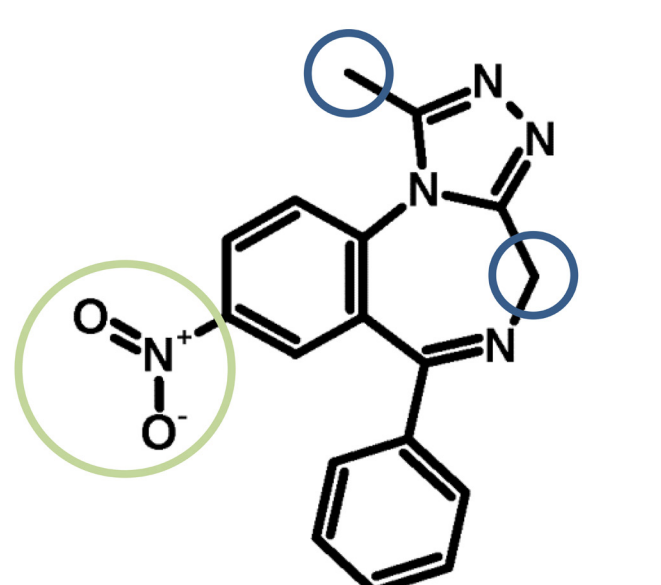
### Metizolam

Two mono-hydroxylated compounds and one di-hydroxylated compound could be identified as the main *in vitro* phase I microsomal metabolites. Assuming similarities in the metabolism of metizolam and etizolam, hydroxylation is most likely to occur at the 2-ethyl moiety or at the 6 position of metizolam. Whichever combination of the aforementioned hydroxylation reactions leads to the detected dihydroxylated metabolite remains unclear.



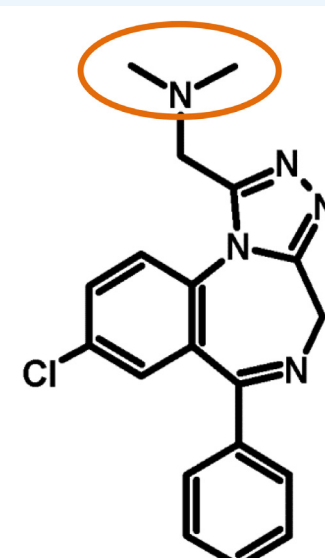
### Nitrazolam

Similar to other nitro-benzodiazepines, reduction of the nitro-moiety leads to the formation of 8-aminonitrazolam. Additionally, one monohydroxylated metabolite was detected, with hydroxylation most likely at the 4- or  $\alpha$ -position of the molecule.



### Adinazolam

The detected *in vitro* metabolites after pHLM incubation, N-desmethyladinazolam and N-didesmethyladinazolam, were in accordance with the main metabolites described in the literature.



Detailed analytical information can be found: Moosmann *et al.* Characterization and *in vitro* phase I microsomal metabolism of designer benzodiazepines – an update comprising adinazolam, cloniprazepam, fonazepam, 3-hydroxyphenazepam, metizolam, and nitrazolam. *J Mass Spectrom*, accepted for publication.

## References

- Moosmann *et al.* Designer benzodiazepines: a new challenge. *World Psychiatry*, 2015; 14 (2): 248.
- Venkatakrisnan *et al.* Kinetic characterization and identification of the enzymes responsible for the hepatic biotransformation of adinazolam and N-desmethyladinazolam in man. *J Pharm Pharmacol*, 1998; 50(3): 265.

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## Conclusion

The five benzodiazepines and one thienodiazepine were structurally characterized and their respective main *in vitro* phase I microsomal metabolites tentatively identified. Future studies should include comparison of the identified metabolites with metabolites formed *in vivo* as well as assessment of basic pharmacokinetic data. Certainly, all described metabolites are prone to undergo phase II metabolic transformations *in vivo*, such as *O*- and *N*-glucuronidation, and acetylation of the amino moiety of the respective metabolites of cloniprazepam, fonazepam and nitrazolam. Formation of licensed benzodiazepines (clonazepam after uptake of cloniprazepam) and the sale of metabolites of prescribed benzodiazepines (fonazepam (identical to norflunitrazepam) and 3-hydroxyphenazepam) pose the risk of wrong interpretation of analytical findings. Consequently, analytical methods should cover a wide range of benzodiazepines and their metabolites.

