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HYPHA DISCOVERY

Metabolites and molecules for tomorrow's drugs

# Drug and metabolite stability studies

## Stability evaluation of parent drug and metabolite formation in fecal incubations

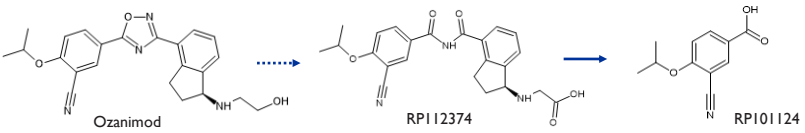
Structural moieties of some drug compounds may be susceptible to chemical reaction in the gut environment, or to biotransformation by microbes in the intestine to gut-specific metabolites. This can be significant enough that resulting metabolites are absorbed and observed as circulating metabolites. Well known is the gut microbiota mediated biotransformation of ozanimod. Ozanimod and an intermediate oxidized metabolite undergo reductive cleavage and hydrolysis resulting in oxadiazole ring scission by anaerobic bacteria in the gut to generate a major metabolite in plasma.

Metabolites of drugs may also be vulnerable to further biotransformation, such as the well known deconjugation of glucuronides by β-glucuronidases produced by bacteria in the gut. This action can result in release of the parent drug in the gut, and significant enterohepatic recirculation.

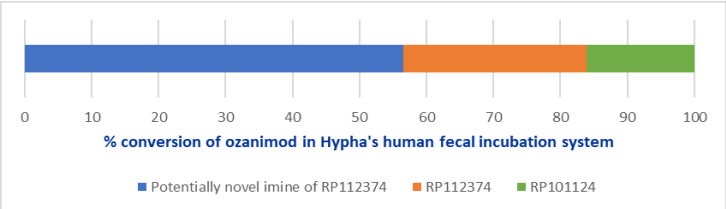
Hypha can monitor stability of drugs or their metabolites in a fecal environment. This is done over a time course comparing incubations in live and heat treated fecal samples and buffer, to check for reversion to the parent drug or further biotransformation. New metabolites can be isolated and their structures elucidated by NMR spectroscopy.

## Features

- Validated pooled mixed gender human fecal samples incubated anaerobically.
- In parallel control incubations e.g. sulfasalazine reductive cleavage.
- Time coursing and analysis of parent drugs and metabolite formation / stability.
- Purification of gut metabolites for further testing.
- Structure elucidation of metabolites by cryoprobe NMR.

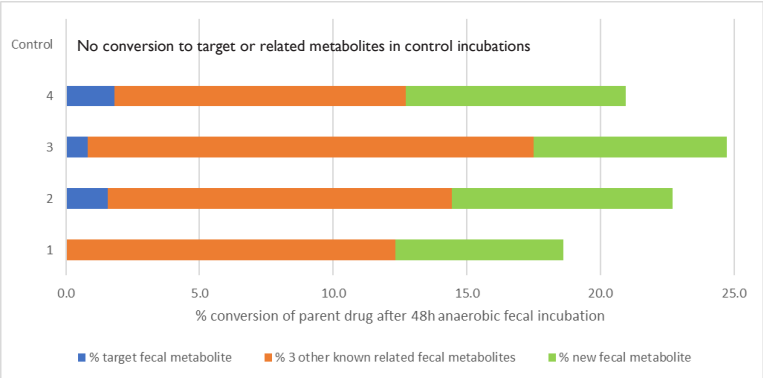



**Reductive cleavage and hydrolysis of ozanimod by gut bacteria to a major circulating metabolite RP101124**




## Client drug stability study

A previously uncharacterised major metabolite was detected in human plasma from SAD and MAD study samples to a 19% total abundance. Hypha conducted a study to investigate whether this ring-rearranged metabolite could be made in the gut by dosing the parent drug in human fecal incubations under anaerobic conditions. The major metabolite was produced under these conditions along with three other known circulating metabolites and the discovery of one new metabolite. Up to 25% conversion of the parent drug was observed after a 48h incubation. As expected, previously characterised oxidized metabolites of the drug were not formed in this system.




**% conversion of parent drug to 5 metabolites after 48h fecal incubations under anaerobic conditions.**

*For more information contact us at*
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### About Hypha Discovery

Hypha Discovery Ltd is a UK-based CRO providing solutions to pharmaceutical and agrochemical R&D partners through the synthesis, purification and identification of metabolites, purification of drug manufacturing impurities and production of natural products. We have an extensive client base and work with many pharma and agrochemical companies worldwide.