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HYPHA DISCOVERY

Metabolites and molecules for tomorrow's drugs

Application of PolyCYPs for metabolite identification from radiolabelled mass balance studies

Hypha's PolyCYPs kits are in routine use by pharma and agchem companies for producing human and other mammalian metabolites. One application involves use of PolyCYPs for creating radiolabelled metabolites for direct comparison with the radio profiles from mass balance and distribution study samples, necessary for regulatory filing. PolyCYPs provides a clean route for scalable access to more of the CYP-derived metabolites observed in these matrices, for definitive MetID and any tox studies deemed necessary. This is especially useful where low concentrations or unstable metabolites in the mass balance sample make structural identification difficult.

One objective of radiolabelled mass balance studies is the identification of significant metabolites. EMA guidance recommend that if a drug metabolite contributes >10% of the AUC of the drug-related material in circulation, then the metabolite should be structurally characterised. Likewise, EFSA guidance for pesticides necessitates metabolism studies, including requiring studies on relevant stereoisomers.

In this application a ^{14}C radiolabelled agrochemical compound was incubated against 8 PolyCYPs enzymes and the metabolites compared with those detected in goat faeces as part of a radiolabelled mass balance study. High resolution LC-MS/MS showed oxidation of cycloalkyl and aromatic carbons of the compound by the PolyCYPs enzyme giving the best conversion for this compound. Metabolites in this PolyCYPs sample matched to the radio labelled metabolites in the goat faeces sample, thereby providing an immediate route to access and scale-up the goat CYP-derived metabolites.

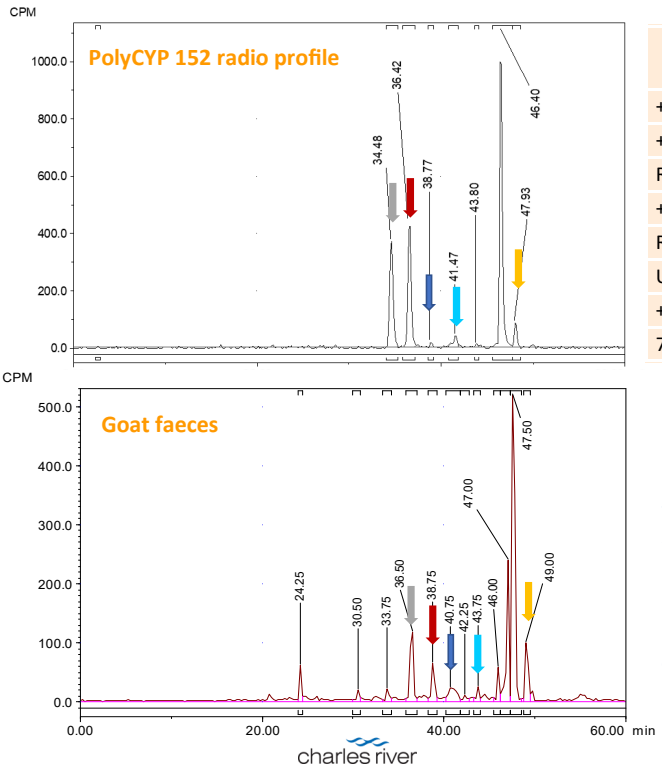
We produce, scale-up and purify phase I and II metabolites using microbial biotransformation, mammalian tissue fractions, recombinant enzymes and chemical synthesis:

- **For DMPK / ADME / TOX**
- **For Met ID**
- **As standards for quantitation**
- **For bioactivity testing**
- **For stability studies**

Proven Reactions

Methyl hydroxylation
Methylene hydroxylation
Methine hydroxylation
Aromatic hydroxylation
***N*-oxidation**
***N*-methylation**
***N*-dealkylation**
***N*-acetylation**
***O*-dealkylation**
Carbonyl reduction
Heterocycle oxidation via aldehyde oxidase
Aromatic *O*-glucuronidation
Aromatic *N*-glucuronidation
Non-aromatic *O*-glucuronidation
Non-aromatic *N*-glucuronidation
Acyl-glucuronidation
Other glycosidations (AgChem)
***N*-sulfation**
***O*-sulfation**
Thiol conjugation (GSH/NAC)
Transamination
Amino acid conjugations
Sequential reactions e.g. hydroxylation & glucuronidation

Radiometric analysis and comparison of the best PolyCYPs sample with a sample from goat faeces.



Name	Rt (min)	ROI (%)
+16 Da (aromatic)	34.48	21.88
+16 Da (aliphatic)	36.42	23.74
Region 3	38.77	0.79
+ 16 Da (aliphatic)	41.47	2.78
Region 5	43.80	0.49
Unchanged Parent	46.40	46.44
+ 16 Da (unknown)	47.93	3.88
7 Peaks		100.00

Subsequent LC-MS/MS analysis showed matching of metabolites in the radiolabelled goat faeces sample with the metabolites formed by PolyCYP 152. Therefore PolyCYP 152 can be used to access and scale-up these goat metabolites.

For more information contact us at mail@hyphadiscovery.co.uk

About Hypha Discovery

Hypha Discovery Ltd is a UK-based microbial biotechnology company providing solutions to pharmaceutical and agrochemical R&D partners through the production of mammalian and microbial metabolites, as well as specialising in microbially-derived chemicals.