

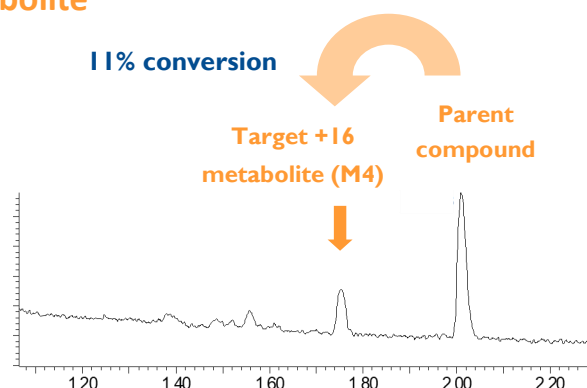
## PolyCYPs Case Study

### Rapid scale-up and purification of a hydroxylated metabolite

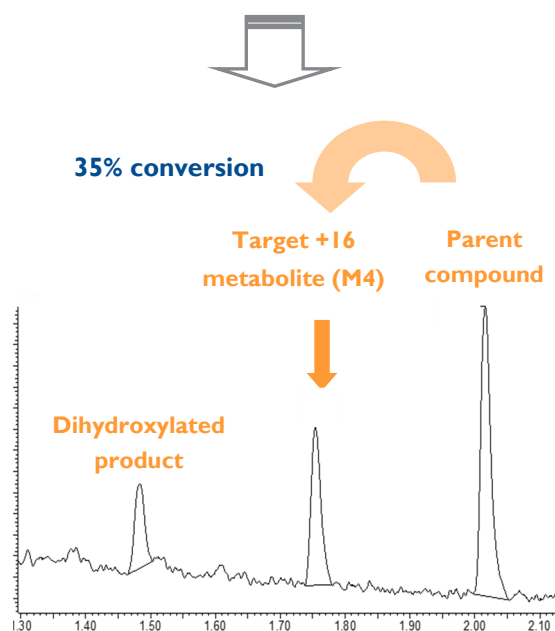
In this case study at least 2 mg of a monohydroxylated metabolite (M4), originally observed in rat liver microsomes, was required by a US pharma company. Screening of the parent compound against 22 PolyCYPs enzymes and 23 microbes revealed the production of two main monohydroxylated products, one of which was M4. M4 was best produced by PolyCYPs 152 and 359, as well as by bacterial species 45. The other monohydroxylated metabolite was produced by a different PolyCYPs isoform, (PolyCYP 350) and bacterial species 1.

Scale-up of PolyCYP 152 was selected as the quickest route for producing mg quantities of M4, prior to which a follow-up dose escalation study performed in 96-well plates showed that the **substrate loading could be increased from 100 mg/L to 300 mg/L** to provide higher volumetric yield of M4.

The scale-up reaction provided a higher conversion than the 11% conversion seen at the screening stage, **increasing to 35% total conversion**. A total of 20.1 mg M4 was purified from the scale-up material using two rounds of HPLC purification. The metabolite was determined to be >97% pure by LC-UV-ELSD, and this was confirmed by <sup>1</sup>H NMR. Lyophilised M4 was supplied to the client together with a Certificate of Analysis within 22 days from receipt of order, exemplifying the short timelines achievable using PolyCYPs to access CYP-derived metabolites.



HPLC analysis of the PolyCYP 152 screening reaction at 100 mg/L substrate loading



HPLC analysis of the optimised scale-up reaction using PolyCYP 152 at 300 mg/L substrate loading

### PolyCYPs scale-up options

| Scale-up vials                      | Bulk enzyme @ Hypha   | Fermentation @ Hypha   |
|-------------------------------------|---|--|
| 1 vial per 1 mg substrate           | Crude enzyme prep   | Whole cell scale-up using streptomycete clone or the originating wild type microbe |
| 10 ml reaction volume per vial      | 100ml - multi litre reaction volume   |  |
| For 100s µgs to low mg requirements | 2-4 weeks for provision of reaction extract +/- purification of metabolites | Yield could be different or altered side products                                  |
| Immediate availability              |   | 6-7 wks from receipt of substrate  |

100s µg to low mg

100s mg to grams

Optional structure elucidation by NMR and provision of Certificate of Analyses by Hypha

PolyCYPs are an integral part of Hypha's newly launched One-Stop Metabolite Shop, which enables clients to access even the most difficult-to-synthesise metabolites using a variety of biological and/or chemical techniques. Hypha are also able to purify metabolites from biological matrices such as urine, and elucidate structures using cryoprobe NMR spectroscopy.