



Contact: julia.shanuwilson@hyphadiscovery.com

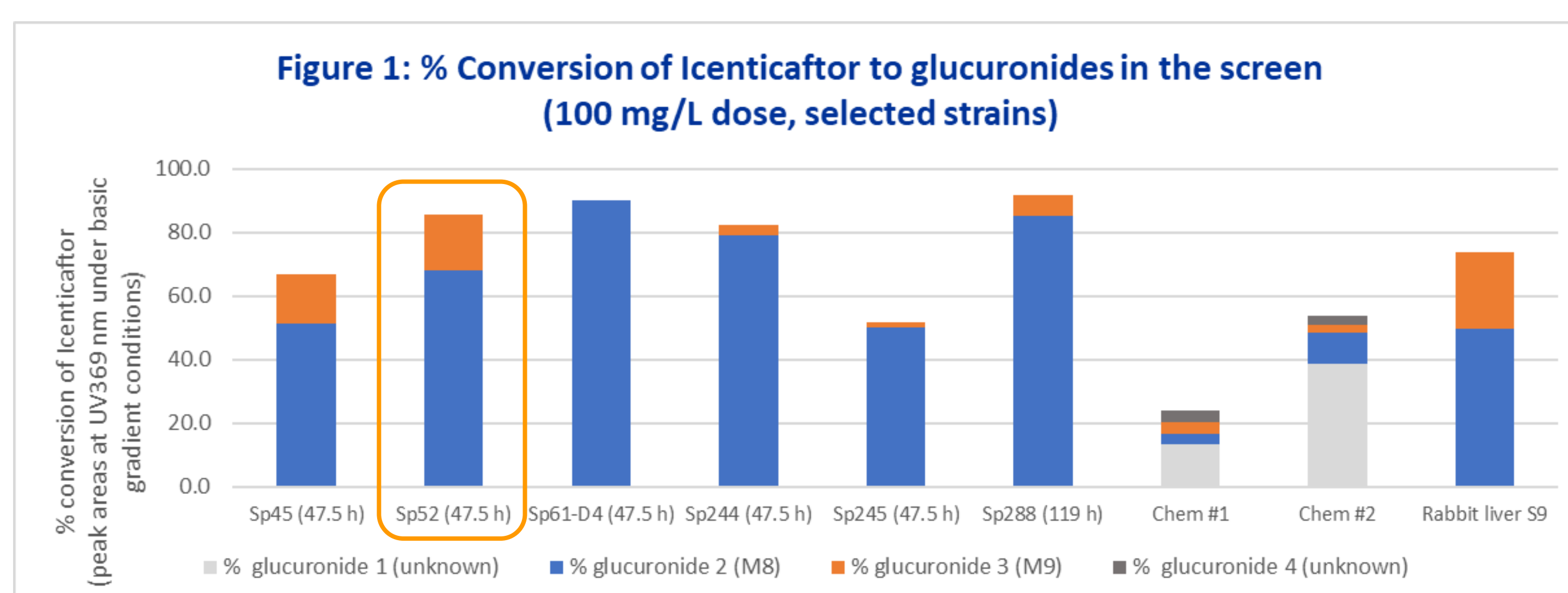
Abstract: Icenticaftor (QBW251) is a potentiator of the CFTR protein which was under development for treatment of COPD and chronic bronchitis by Novartis. In humans it is extensively metabolised. Direct N-glucuronidation and O-glucuronidation formed the most abundant human metabolites M8 and M9, respectively. [1]. Initial amounts of M8 and M9 were made for MetID purposes using biotransformation with rabbit liver S9 fraction [1]. For further testing and analysis method calibration in later phase studies, more material was required of M8 and M9, and also of M5, which is formed following demethylation of icenticaftor and subsequent O-glucuronidation. Alternative scalable methods were subsequently used to generate this material using a combination of late-stage chemical synthesis (M5) and microbial biotransformation (M8 and M9). This poster describes the application of microbial biotransformation [2,3] to make scalable amounts of M8 and M9.

Glucuronide biosynthesis

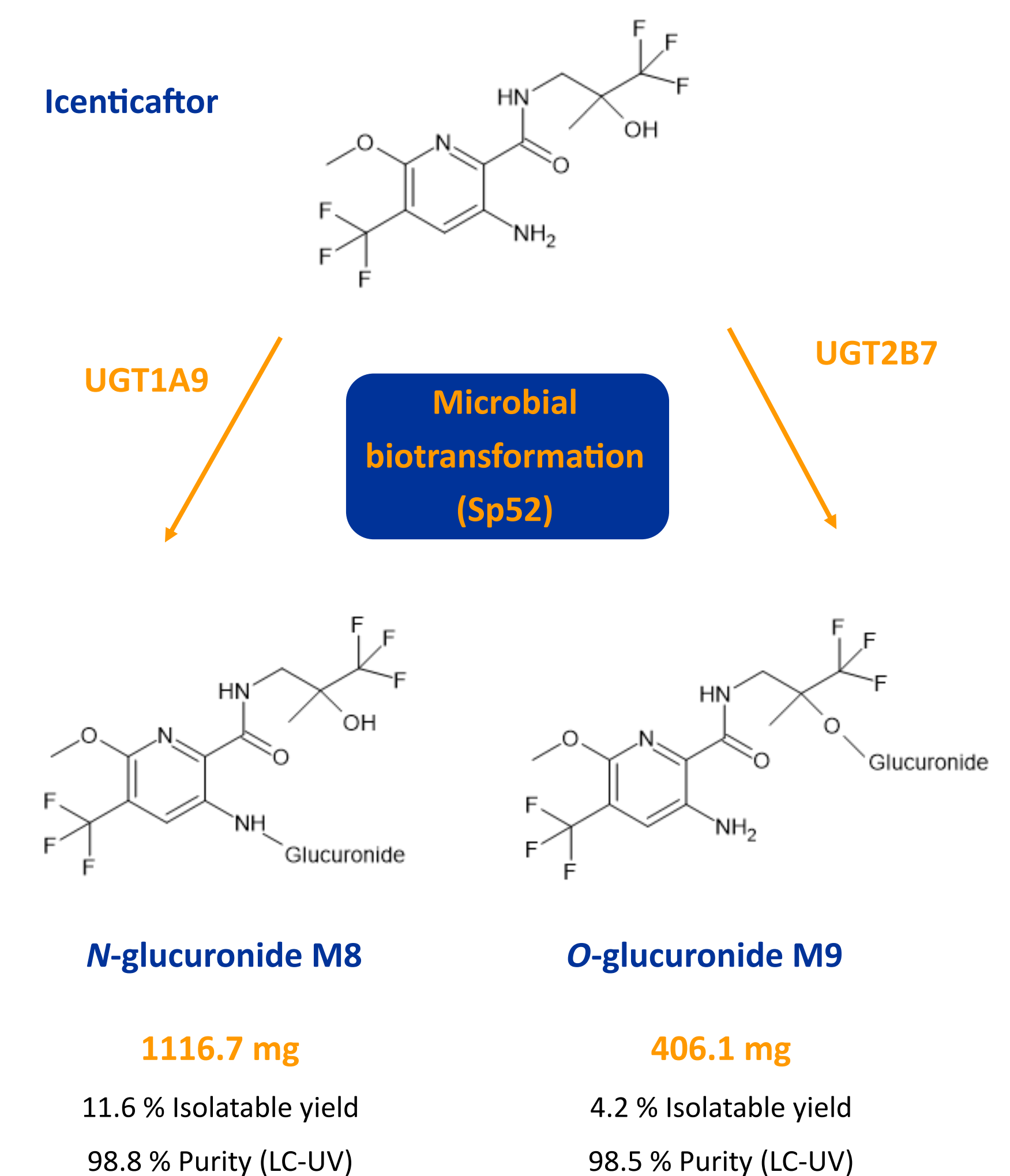
Screening

A prioritized panel of 24 microorganisms and several chemical synthesis conditions were screened to look for the production of glucuronides of Icenticaftor and compared to those observed in rabbit liver S9 incubations. A biosynthetic route was found to be superior for production of both glucuronides with several strains shown to be capable of glucuronidation (Figure 1).

Only two strains could produce both glucuronides observed in the rabbit liver S9 incubations at scalable yields. Strain Sp52, a bacterial strain of the genus *Streptomyces*, could make both M8 and M9 at an 86% total conversion of a 100 mg/L substrate dose, mimicking the action of human UGTs UGT1A9 and UGT2B7 in synthesis of M8 (N-glucuronide) and M9 (O-glucuronide), respectively.



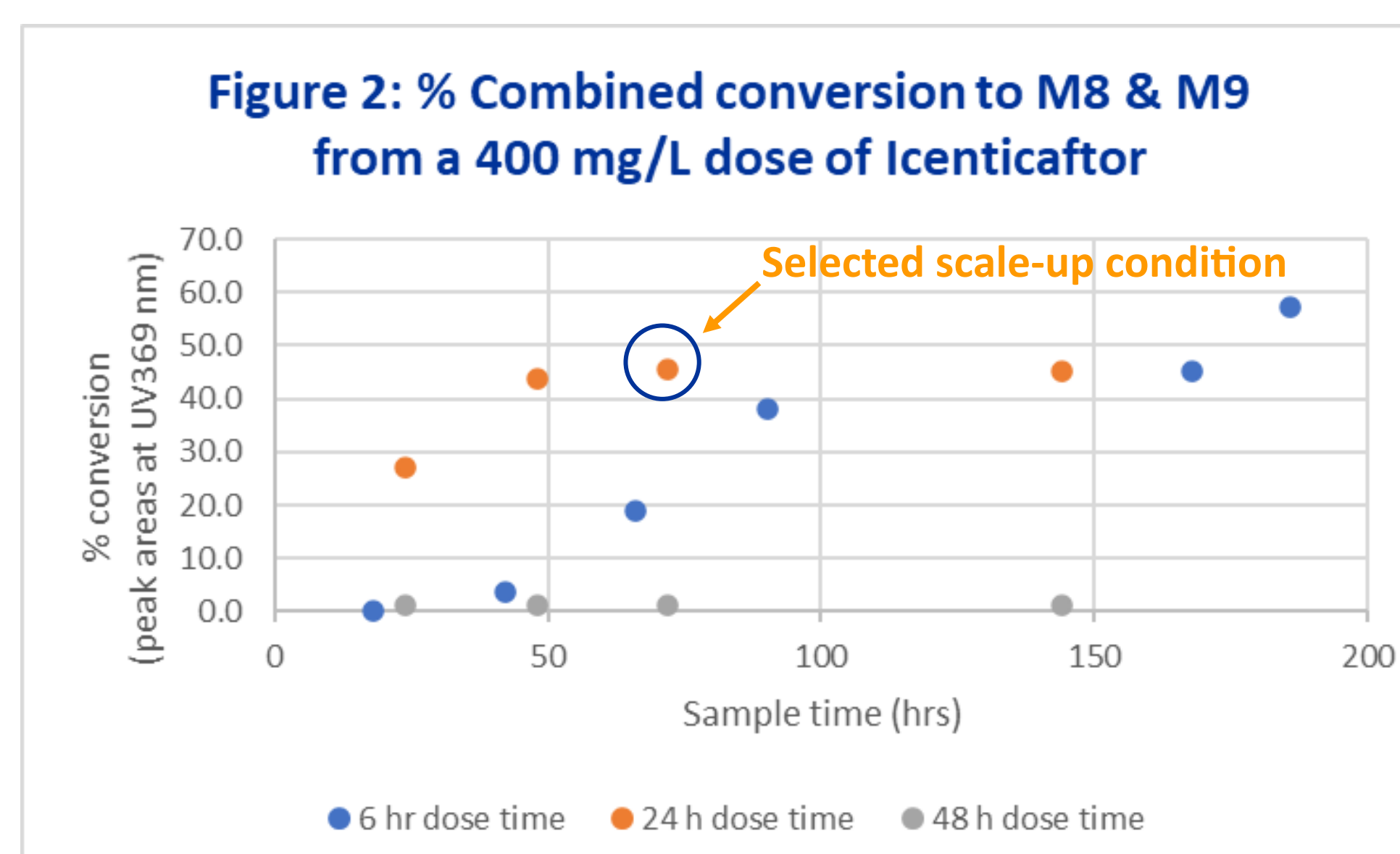
Provision of major direct glucuronides of Icenticaftor by microbial biotransformation using a *Streptomyces* strain (Sp52)



Dose Escalation and Scale-up

To reduce the volume of the reaction needed to produce at least 220 mg of each metabolite, a dose escalation experiment was performed comparing the original 100 mg/L dose with doses up to 600 mg/L parent dose, each at addition times of 6 h, 24 h and 48 h. Results showed that although almost complete conversion could be obtained under scale-up conditions with 100 mg/L Icenticaftor dosed at 6 h, 400 mg/L of Icenticaftor dosed at 24 h with harvesting after 72 h gave the best overall volumetric results (Figure 2). The ratio of glucuronides produced was 76% M8 to 24% M9, which is similar to the ratio observed in the screening reaction.

Biotransformation reactions were scaled up via 3 x 8 L sub-batches dosed with a total of 9.6 g Icenticaftor which, after extraction and two-stage orthogonal preparative HPLC purification, provided 11.6% (1116.7 mg) M8 and 4.2% (406.1 mg) M9. Purification minimized any degradation of M8 which is known to be unstable under acidic conditions.



Conclusion

- Both M8 and M9 were produced by one microbe, mirroring the major circulating glucuronides seen in humans and in a similar ratio to M8 and M9 produced in human hepatocyte incubations (84% M8 : 16% M9 [1]).
- Increasing the dose of Icenticaftor four-fold enabled the volume of the scale-up reaction to be halved to achieve the amounts purified.
- A lower than usual purification yield (34%) was obtained, possibly due to the close elution of M8 and M9 and the high purity needed.
- Following purification the certified metabolites were delivered to Novartis and used as calibration standards in further studies.

References

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- [2] Identification and Biosynthesis of an N-Glucuronide Metabolite of Camonsertib. Robert Papp, Laird Trimble, Adrian J. Fretland, Ravi Manohar, Richard Phipps, Lisbet Kvaerno, Alexander L. Perryman, Gregory Reynolds and W. Cameron Black (2024). *Drug Metab Dispo*, 52 (5): 368-376. DOI: <https://doi.org/10.1124/dmd.123.001611>
- [3] Microbial biotransformation – an important tool for the study of drug metabolism. Salter, R., Beshore, D. C., Colletti, S. L., Evans, L., Gong, Y., Helmy, R., ... Martin, I. J. (2018). *Xenobiotica*, 49 (8): 877-886. DOI: <https://doi.org/10.1080/00498254.2018.1512018>