AN INVESTIGATION INTO THE USE OF 4-METHYLUMBELLIFERONE, p-NITROPHENOL AND 1-NAPHTHOL AS SUBSTRATES FOR RECOMBINANT HUMAN SULT1B1 EXPRESSED IN E. coli.

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ABSTRACT
It is well known that SULT1B1 acts on thyroid hormones but the range of exogenous substrates for this enzyme has not been fully explored. We have therefore investigated the use of 4-methylumbelliferone and 1-naphthol as substrates for determinations of endogenous human SULT1B1 activity, and 4-nitrophenol as a probe for recombinant SULT1B1.

INTRODUCTION
Sulfotransferases are important Phase II drug metabolising enzymes, catalysing the sulfation of xenobiotics, hormones and neurotransmitters. SULT1B1 is one of seven sulfotransferases. It is responsible for the sulfation of thyroid hormones, 1-naphthol and p-nitrophenol. While SULT1B1 has been shown to sulfate p-nitrophenol, an alternative substrate used by us for the assay of this enzyme. We have investigated two substrates, 1-naphthol and p-nitrophenol.

METHODS AND REAGENTS
Chemicals
Chemicals and substrates were purchased from Sigma-Aldrich for the determination for in vitro assays. All other chemicals were purchased from Fisher Scientific or VWR. Recombinant human SULT1B1 was expressed in E. coli and purified from inclusion bodies using a procedure involving denaturation in 8M urea, followed by precipitation with ammonium sulfate, and refolding of the recombinant protein in the presence of a chaperone. The recombinant protein was then isolated from E. coli by soluble fractionation. All of the substrates were sulfated by SULT1B1 and, in each case, the formation of metabolite was found to be linear with respect to time for at least 5 minutes and with protein concentration up to 20 µg/200 µl.

RESULTS
All of the substrates were sulfated by SULT1B1 and, in each case, the formation of metabolite was found to be linear with respect to time for at least 5 minutes and with protein concentration up to 20 µg/200 µl.

CONCLUSIONS
As the Km and Vmax values for SULT1A1*1 with 4MU differ from those for SULT1B1, it allows us to distinguish between the two enzymes (Average values for SULT1A1*1, Km : 1.3 µM, Vmax : 9.5 pmol/min/µg protein). Although there was slight substrate inhibition present at concentrations of 4MU above 80 µM, this does not affect our assays as the concentrations used are run at a far lower substrate concentration.

REFERENCES

Fig 1. Linearity with time for SULT1B1 with p-nitrophenol as substrate
Fig 2. Linearity with protein concentration for SULT1B1 with p-nitrophenol as substrate
Fig 3. Hanes plot for pNP assay with SULT1B1
Fig 4. Fit of data to Michaelis-Menten equation for pNP assay with SULT1B1
Fig 5. Hanes plot for 4MU assay with SULT1B1
Fig 6. Fit of data to Michaelis-Menten equation for 4MU assay with SULT1B1
Fig 7. Plot of [S] against v for 1-naphthol assay with SULT1B1