RECOMBINANT HUMAN CYP1A2 CATALYSES THE O-DEMETHYLATION OF 7-METHOXYCOUMARIN

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Abstract
1. HLC conditions were established for the separation of 7-methoxycoumarin, 7-hydroxycoumarin and 7-hydroxycoumarin bis-glucuronide (7-HCG) from each other.
2. The 7-HCG glucuronides, generated from a commercial source, were cleaned up by exhaustively extracting to remove a small amount (0.2-0.3%) of USP 7-methoxycoumarin (USP 7-MC)
3. In a pilot experiment, recombinant human CYP1A2 was shown to catalyse 7-methoxycoumarin O-dealkylation (7-MOC activity).
4. Inclusion conditions were optimised to give linear formation of product with respect to incubation time and CYP concentration, at a constant concentration of 14 μM 7-MOC in CYP Assay Batteries, the reaction was linear for 30 min at 37°C and pH 7.4. 5. Product analysis of CYP1A2 Batteries was characterized by ESI-MS/MS (30–90 m/s) and at pH 7.4 and 8.0. (Corresponding kinetic parameters for the recombinant CYP1A2 O-dealkylation (7-MOC activity) were kcat = 0.1 and 0.2 nmol/mg of CYP and kcat/km = 0.3 and 0.5 μM, respectively).

Introduction and Aim
The O-demethylation of 7-MOC (TC) is catalysed by multiple recombinant human CYPs. In contrast, very little is known about the role of human CYPs in the O-demethylation of other flavonoid compounds. This is a somewhat perplexing, given the wealth of such competitive data relating to the O-demethylation of the flavonoids.

The aim of this study was therefore to establish an assay for the determination of CYP1A2 O-dealkylation (7-MOC) activity, and subsequently to investigate a possible role for human CYP1A2 in catalysing this reaction.

Results
A) A 7-HLC gradient separation of 7-MOC, TC and 7-HCG was established (Figure 1). Retention times for the three compounds were 4.7 min (7-HCG), 5.4 min (TC) and 15.3 min (TC). These HPLC conditions could also be used to separate O-monoglucuronidated metabolites from 7-HCG (data shown) allowing this compound to be used as an internal standard. Efficient, levels of detection for 7-HCG and TC, taken as the three times signal noise ratio were 10 and 20 ng, respectively.

The analysis of the fluorescence method revealed a small amount of 7-MOC (<0.2%), present as its monoglucuronidated metabolite (7-MOC). At a concentration of 7-MOC, the absorbance at 240 nm was subtracted, 0.001 absorbance units were read, a calibration curve was fitted, with a recovery of 100%.

Conclusions
1. TC, 7-MC and TC can be separated by reversed phase HPLC, using an acetonitrile gradient. Fluorescence detection allows the detection of very small (<0.2%) amounts of the TC metabolites.
2. Recombinant human CYP1A2 demonstrated 7-MOC activity. The intrinsic clearance of TC is almost twice that of TC for the O-demethylation reaction.
3. Investigation into the O-demethylation of TC by a panel of human CYPs could provide useful data, especially when combined with the corresponding data generated for TC (1).

References