PACLITAXEL (TAXOL) HYDROXYLATION ASSAY

This assay measures the P450-dependent conversion of the anti-cancer agent paclitaxel (i.e. **Taxol; TAX**) to its 6α-hydroxylated metabolite, 6-hydroxypaclitaxel (**6α-OHT**). Paclitaxel 6α-hydroxylation occurs at C6 of the taxane ring (see below), and is catalyzed exclusively by CYP2C8. Paclitaxel is also hydroxylated at the C3' phenyl moiety of the molecule by CYP3A4(5). Although CYP2C8 is not a polymorphic P450 enzyme, metabolism of paclitaxel exhibits marked interindividual variation due to the large variation in CYP2C8 expression among individuals.

I. REAGENTS

A. 100 mM KPO_4 buffer, pH 7.4, at room temperature.

B. 5 mM Paclitaxel — **MW = 854;** Prepare by dissolving 4.3 mg TAX (solid) in 1.0 ml MeOH containing 0.1% acetic acid. **Store at -20°C**. [Addition of 0.1% acetic acid will stabilize solution].

C. 10 mM NADPH — Prepare by dissolving 4.7 mg in 0.5 ml Buffer A - enough for 20 assay tubes.

D. Ethyl Acetate
D1. Acetonitrile (ACN)

E. Methanol (MeOH)

F. 25 µM **6α-OHT** (MW = 870) — Prepare by adding 11 µl of 1.15 mM **6α-OHT** (1 mg authentic **6α-OHT**/ml MeOH) to 489 µl MeOH.

II. PROCEDURE

1. Pipette appropriate amount of Reagent A into 1.5 ml Eppendorf-type microfuge tubes, keeping in mind that the final assay volume is **0.25 ml**. Place tubes in an ice bath.

2. Add human liver microsomes (0.1-0.2 nmol microsomal P450) to the tubes.

3. Add 5 µl of Reagent B to each tube, giving final TAX concentration of **100 µM.**

4. Add 25 µl of Reagent C to the appropriate tubes, vortex, and place tubes in shaking water bath at 37°C. **DO NOT ADD** Reagent C to incubation tubes that will be used as blanks or for standards.

5. Reactions are terminated after **30 min** by adding 1.0 ml of Reagent D, vortexing the tubes, and placing them in ice. After all reactions have been stopped, vortex tubes for 5 min using the Multiple Vortexer.

6. Centrifuge tubes at 15,000 rpm for 5 min in the Heraeus Microfuge to separate the organic and aqueous layers. Transfer **0.70 ml** of the organic (upper) phase to a 10 x 75 mm disposable glass tubes, and evaporate sample to dryness at room temp using the N_2-EVAP nitrogen evaporator.

7. Add 60 µl of Reagent E to each tube, cover with plastic cap, vortex briefly, and sonicate for 5 min in a bath-type sonicator to completely resolubilize residues. Process samples by HPLC as described below or store @ -20°C until HPLC analysis can be performed.

☞**NOTE A** - Assay blanks contain all components except NADPH (Reagent C). Standard curves are constructed by adding 2, 5, 10, 20 and 30 µl of Reagent E (equivalent to 50, 125, 250, 500 and 750 **pmol** 6α-OHT) to the assay tubes with NADPH OMITTED, and performing assay as described above.

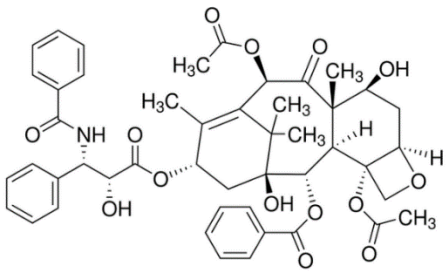
III. HPLC ANALYSIS CONDITIONS FOR 6α-OHT & TAX

Column:	Phenomenex Kinetex column (4.6 x 150 mm) w/CrudCatcher Guard Column
Mobile Phase:	Solvent A – H_2O
	Solvent B – ACN
Flow Rate:	1.0 ml/min
Column Temp:	Ambient
Sample Temp:	Ambient
Peak Detection:	**232 nm**
Run Time:	21 min
Injection Volume:	**30 µl**

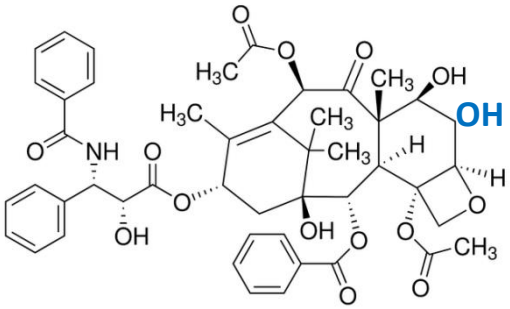
KINETEX Column

(min)	(A %)	(B %)	(ml/min)	(gradient)
Initial	60	40	1.0	
10.0	25	75	1.0	6
10.1	60	40	1.0	1

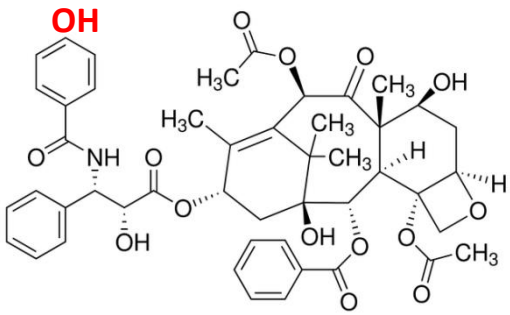
Using these HPLC conditions, **6α-OHT** at 5.4 min while the parent compund **TAX** elutes at 6.7 min, respectively.



PACLITAXEL

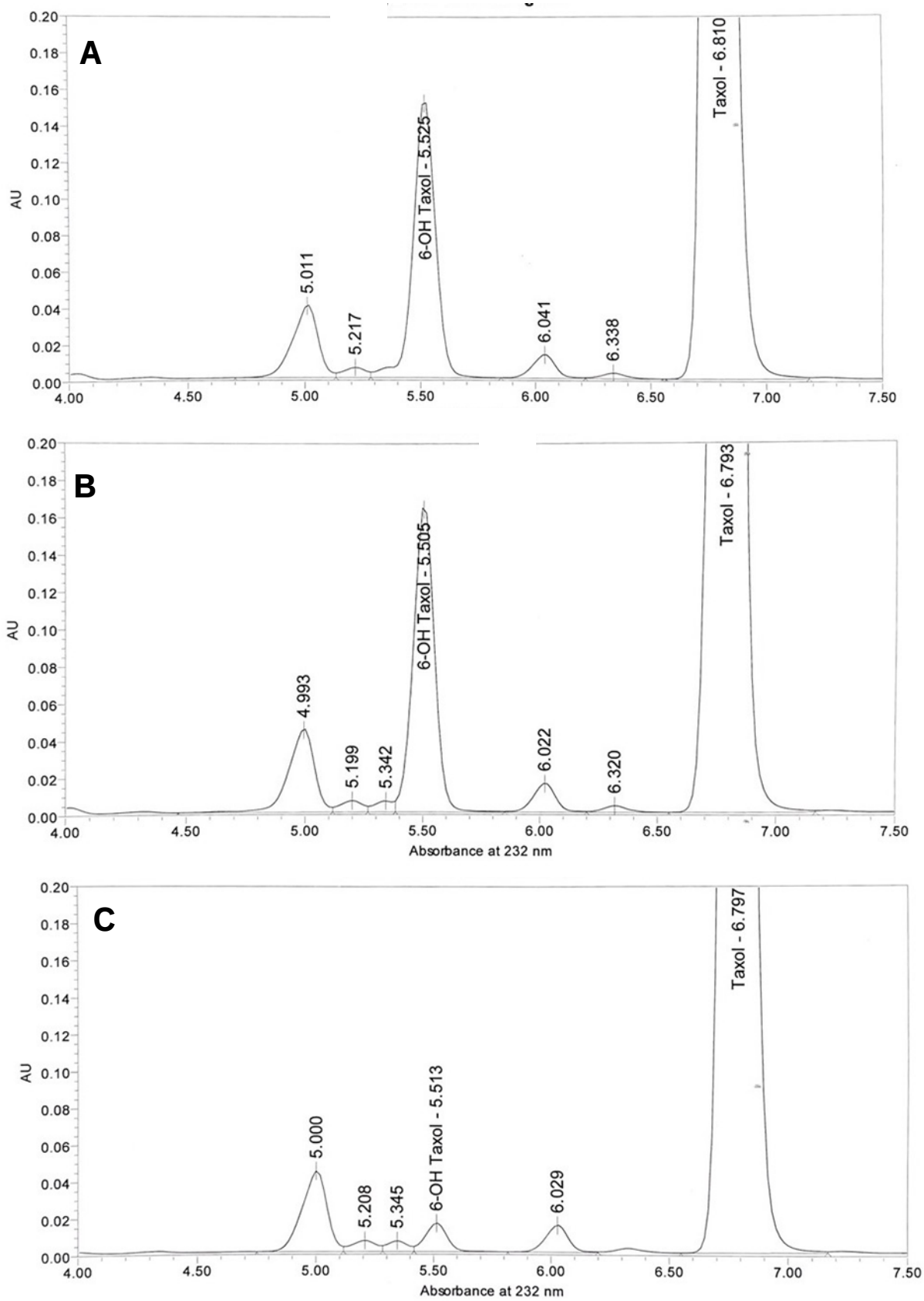


6α-HYDROXYPACLITAXEL
CYP2C8



C3'-HYDROXYPACLITAXEL
CYP3A4

PACLITAXEL - CYP2C8



HPLC analysis of paclitaxel 6α-hydroxylation by human liver microsomes. Paclitaxel (TAX; 100 µM) was incubated with human liver microsomes (0.24 mg protein, 0.1 nmol aggregate P450) for 30 min at 37°C in the presence of NADPH. plus 0.5 mg anti-CYP1A2, 0.05 mg anti-CYP2C8 mAb or 0.5 mg control (preimmune) IgG. TAX was then resolved from the 6-hydroxylpaclitaxel (6α-OHT) metabolite by reversed-phase HPLC as described above using a Waters Alliance unit equipped with a model 2690 separation module and a model 2487 UV/VIS detector. A Phenomenex C18 column (4.6 mm x 15 cm, 5 µm particle size) was utilized. Gradient elution was performed with a mobile phase consisting of acetonitrile:H_2O (see above) employing a flow rate of 1.0 ml/min, and the column eluates were continuously monited for UV absorbance at 232 nm. Under these conditions, TAX and 6a-OHT exhibited retention times of 5.4 and 6.7 min, respectively; the total sample analysis time was 21 min. Formation of 6a-OHT was determined by comparison of peak areas with those of analytical metabolite standards.

a) TAX + liver microsomes + NADPH + Preimmune IgG
b) TAX + liver microsomes + NADPH + Anti-CYP1A2 IgG
c) TAX + liver microsomes + NADPH + Anti-CYP2C8 mAb