BUPROPION HYDROXYLATION ASSAY

This assay measures P450-dependent oxidation of the oral anti-depressive agent bupropion (Wellbutrin® or Zyban®)(BUP) to its hydroxylated metabolite hydroxybupropion (OH-BUP). High-affinity BUP hydroxylation in human liver is catalyzed exclusively by CYP2B6.

I. REAGENTS NEEDED

A) 100 mM Potassium Phosphate buffer, **pH 7.4**, at room temperature.

B) 10 mM BUP MW = 276.2	Prepared by dissolving 5.0 mg BUP in 1.8 ml MeOH Buffer/BUP solution is made by adding 100 µl 10 mM BUP to 3.90 ml Reagent A (enough for 18 assay tubes) Gives final conc of 250 µM BUP.
C) 10 mM NADPH	Prepare by dissolving 4.7 mg in 0.5 ml Buffer A – enough for 20 assay tubes.
D) Acetonitrile	
E) 100 µM OH-BUP MW = 255.7	Prepare by dissolving 1 mg OH-BUP solid/0.4 ml MeOH to give 10 mM OH-BUP. Then, dilute 50 µl of 10 mM OH-BUP with 4.95 ml of Reagent A to give 100 µM OH-BUP (See Note A below)

II. PROCEDURE

1. Pipette appropriate amount of Reagent A containing **250 µM BUP** 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 (0.05 - 0.1 nmol microsomal P450) to the tubes.

3. 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 metabolite standards.

4. Terminate reactions after **30 min** by adding 100 µl of Reagent D, and vortexing well.

5. Centrifuge tubes at 15,000 rpm for 10 min in the Heraeus Microfuge to precipitate protein. Then, transfer 200 µl of the clear supernatant to 10 x 75 mm disposable glass tubes.

6. Transfer 100 µl of the supernatants from #5 above to autosampler vials w/150 µl glass inserts, seal with caps, and subject to HPLC or store @ -20°C until analysis is performed.

NOTE A - Assay blanks contain all components except NADPH (Reagent C). Standard curves are constructed by adding 5, 10, 20, 30 and 50 µl of Reagent E (0.5, 1, 2, and 3 and 5 nmol, respectively) to assay tubes with NADPH OMITTED, and performing assay as described above.

III. HPLC ANALYSIS CONDITIONS FOR BUP & HYDROXY-BUP

Column:	Phenomenex Kinetex column (4.6 x 150 mm) w/Security Guard Column
Mobile Phase:	Solvent A – 0.1% Phosphoric Acid
	Solvent B – ACN
Flow Rate:	1.0 ml/min
Column Temp:	Ambient
Sample Temp:	Ambient
Peak Detection:	214 nm
Run Time:	8 min
Injection Volume:	50 µl

KINETEX Column

(min)	(% A)	(% B)	(ml/min)	(gradient)
Initial	80	20	1.0	--
8	80	20	1.0	--

Using these HPLC conditions, Hydroxy BUP elutes at **3.4 MIN** while the parent compound BUP elutes at **5.5 min** (see attached chromatogram).

CITATIONS

Faucette SR, Hawke RL, LeCluyse EL, Shord SS, Yan B, Laethem RM, Lindley CM: Validation of bupropion hydroxylation as a selective marker of human cytochrome P450 2B6 catalytic activity. *Drug Metab Dispos* 28:1222-1230, 2000.

Pearce RE, Gaedigk R, Twist GP, Dai H, Riffel AK, Leeder JS, Gaedigk A: Developmental expression of CYP2B6: a comprehensive analysis of mRNA expression, protein content and bupropion hydroxylase activity and the impact of genetic variation. *Drug Metab Dispos* 44:948-958, 2016.

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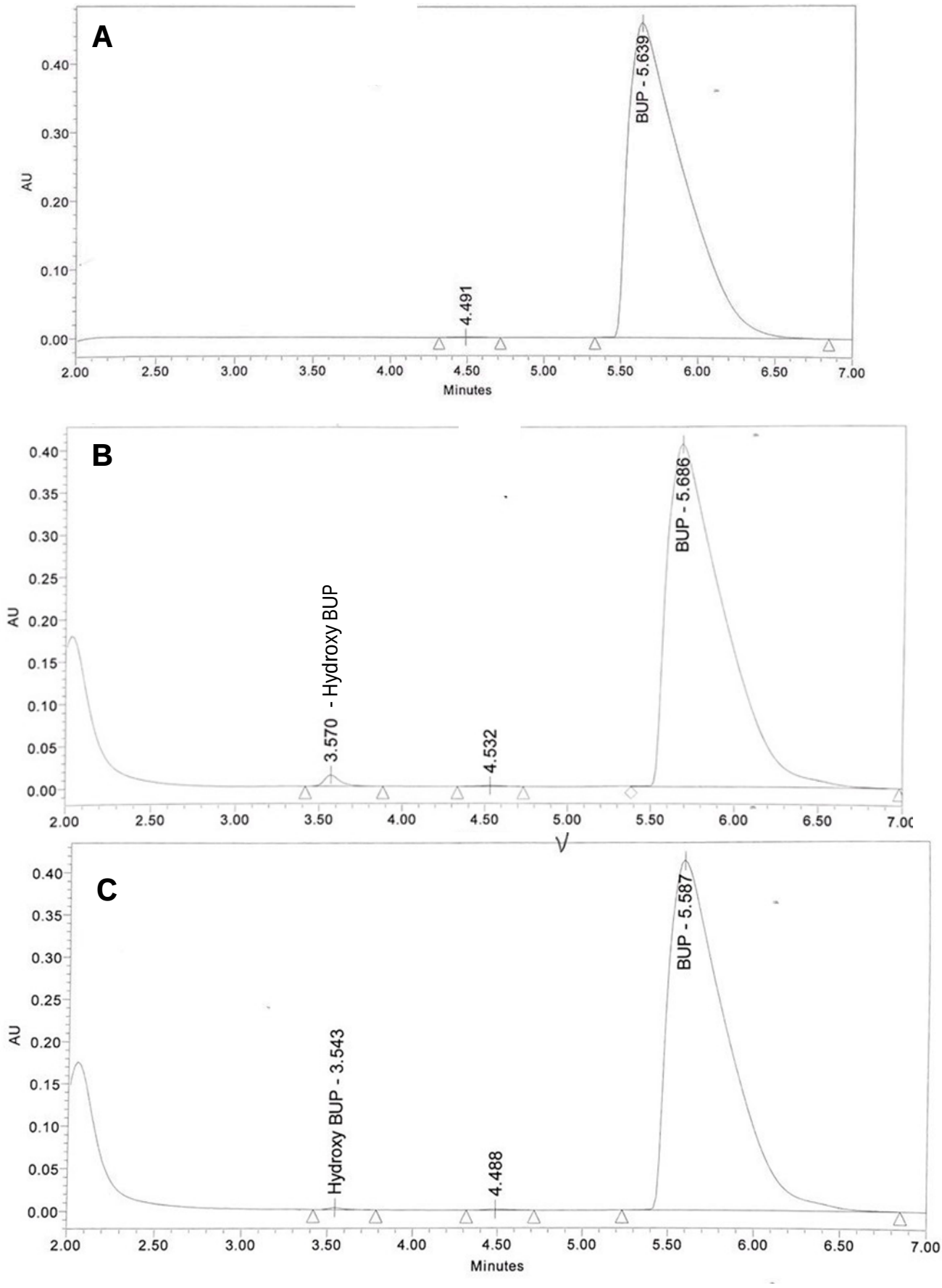

HPLC analysis of bupropion 6-hydroxylation by human liver microsomes. Bupropion (BUP; 250 µM) was incubated with human liver microsomes (0.1 nmol aggregate P450) for 30 min at 37°C in the presence of NADPH. BUP was resolved from the metabolite 6-hydroxybupropion (hydroxy BUP) reversed-phase HPLC according to a method described above, as modified from Faucette et al. (2000). HPLC was performed with a Waters Alliance 2690 HPLC system equipped with a diode array detector. Hydroxy BUP and BUP were resolved using a Phenomenex Kinetex C18 column (4.6 mm x 15 cm, 5 mm particle size). The mobile phase was a 4:1 mixture of 0.1% phosphoric acid and acetonitrile using a flow rate of 1 ml/min, and the column eluate was monited at 214 nm. Under these conditions, hydroxy BUP and BUP had retention times of 3.6 and 5.6 min, respectively; the total sample analysis time was 8 min. Hydroxybupropion formation was quantified by comparison of peak areas with those of analytical standards.

a) BUP + liver microsomes minus NADPH + Preimmune IgG
b) BUP + liver microsomes + NADPH + Preimmune IgG
c) BUP + liver microsomes + NADPH + Anti-CYP2B6 MaB