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 hydroxyP 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.

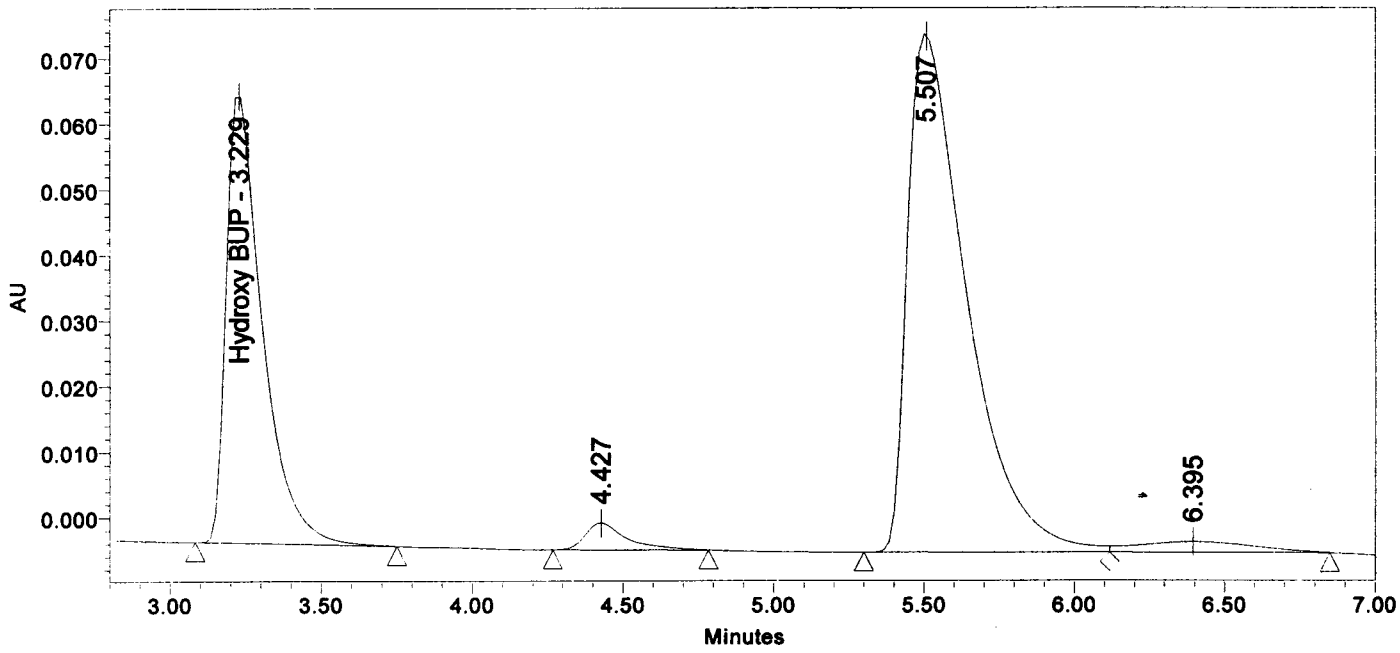
BUP Hydroxylation Report Report

Reported by User: System Project Name: Defaults

SAMPLE INFORMATION

Sample Name:	BUP STDS	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	9/1/2020 9:26:10 PM
Vial:	74	Acq. Method Set:	BUP Isocratic
Injection #:	1	Date Processed:	9/1/2020 11:18:00 PM
Injection Volume:	5.00 ul	Processing Method	BUP Quantitation
Run Time:	8.0 Minutes	Channel Name:	2487Channel 1
Sample Set Name	BUP Hydroxylase Inhibit 090120	Proc. Chnl. Descr.:	

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1	Hydroxy BUP	3.229	566059	68276		
2		4.427	39170	4107		
3		5.507	1078235	79277		
4	BUP	5.873				
5		6.395	43674	1619		