

## Enzyme Assays

### CYP2D6: Assay Conditions for Bufuralol 1'-Hydroxylation

*Bufuralol 1'-hydroxylation is a low  $K_m$ , high turnover assay for human CYP2D6. The apparent  $K_m$  for the CYP2D6-catalyzed reaction is 5-10  $\mu$ M. The turnover number (per unit cDNA-expressed CYP2D6 is 10 to 30  $\text{min}^{-1}$ ). When performing kinetic analyses near the apparent  $K_m$ , protein concentration and incubation time should be chosen to avoid excessive metabolism of substrate. BD Biosciences supplies 1'-hydroxybufuralol metabolite standard (Cat. no. 451034) for quantitation of the assay results.*

#### Solutions

- I. 1 mM Bufuralol (Cat. no. 451034) in 0.1 M potassium phosphate pH 7.4, 1:5 dilution of Cat. no. 451201
- II. 20 mg/ml Glucose 6-phosphate, 20 mg/ml NADP, 13.3 mg/ml  $\text{MgCl}_2 \cdot \text{H}_2\text{O}$  (Cat. no. 451220)
- III. 40 U/ml Glucose 6-phosphate dehydrogenase in 5 mM sodium citrate (tribasic) (Cat. no. 451200)
- IV. 0.1 M Potassium phosphate pH 7.4, 1:5 dilution of Cat. no. 451201
- V. 70% Perchloric acid

#### Incubation Conditions (for 1 ml Final Volume)

- 50  $\mu$ l Solution II.  
10  $\mu$ l Solution III.  
100  $\mu$ l Solution I. (Final concentration 100  $\mu$ M, a saturating concentration)  
xx  $\mu$ l Enzyme. (human liver microsomes or cDNA-expressed)  
840 - xx  $\mu$ l Solution IV.

Mix and prewarm to 37°C all solutions except enzyme. Initiate incubation with the addition of enzyme. After the desired incubation time, stop the reaction by the addition of 100  $\mu$ l of solution V and cool on ice. Centrifuge 12000  $\times$  g for 4 minutes to precipitate protein. Analyze the supernatant for product formation by HPLC separation with fluorescence detection. Recommended range of injection volumes - 10 to 150  $\mu$ l.

#### HPLC Conditions

Mobile Phase A: 30% Acetonitrile, 70% water, 1 mM Perchloric acid (See Note 1)

Isocratic

Column: Nucleosil C18, 4.6 x 250 mm, 5  $\mu$ m particle size (see Note 2)

Temperature: 50°C (see Note 3)

Flow Rate: 1 ml/min

Detector: Fluorescence: Excitation at 252 nm and Emission at 302 nm

Retention Times: 1'-Hydroxybufuralol, 6 minutes; Bufuralol, 20 minutes

#### Note 1

Bufuralol and 1'-hydroxybufuralol are being chromatographed as ion pairs with perchlorate as the counter ion.

#### Note 2

1'-Hydroxybufuralol (Cat. no. 451034) and bufuralol are easily separated and most C18 columns should be adequate for the purpose. However, some adjustment in mobile phase acetonitrile concentration may be desired.

#### Note 3

Column temperature can range from room temperature to 50°C. The use of a controlled, elevated temperature provides greater reproducibility in retention times and lower column back pressures.

#### Reference

Anal. Biochem. (1987) 162, 24