

Enzyme Assays

CYP2C8: Assay Conditions for Paclitaxel 6(alpha)-Hydroxylation

Paclitaxel 6(alpha)-hydroxylation is a low K_m , relatively high turnover assay for human CYP2C8. The apparent K_m for the CYP2C8-catalyzed reaction is 4-8 μ M. 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 6(alpha)-hydroxypaclitaxel metabolite standard (Cat. No. 451656) for quantitation of the assay results.

Solutions

- I. 5 mM Paclitaxel in ethanol
- II. 20 mg/ml Glucose 6-phosphate, 20 mg/ml NADP, 13.3 mg/ml $MgCl_2 \cdot H_2O$, (Cat. no. 451220)
- III. 40 U/ml Glucose 6-phosphate dehydrogenase in 5 mM sodium citrate (tribasic), (Cat. no. 451200)
- IV. 0.05 M Potassium Phosphate pH 7.4, 1:10 dilution of Cat. no. 451201
- V. 100% Acetonitrile

Incubation Conditions (for 1 ml Final Volume)

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|-------------|---|
| 50 ul | Solution II. |
| 10 ul | Solution III. |
| 2 ul | Solution I. (Final concentration 10 μ M, near the limit of solubility in aqueous media) |
| xx ul | Enzyme. (human liver microsomes or cDNA-expressed) |
| 938 - xx ul | 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 300 ul of solution V (per ml) and cool on ice. Centrifuge 12000 x g for 4 minutes to precipitate protein. Analyze the supernatant for product formation by HPLC separation with UV detection. Recommended range of injection volumes: 50 to 100 ul.

HPLC Conditions

Mobile Phase A: 10% methanol (see Note 1)

Mobile Phase B: 100% methanol

Gradient: Initial conditions: 55% B with a linear gradient to 65% B over 20 minutes then hold at 65% B for 5 minutes

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

Temperature: 45°C (see Note 3)

Flow Rate: 1 ml/min

Detector: Absorbance at 230 nm

Retention Times: 6(alpha)-Hydroxypaclitaxel (Cat. no. 451656), 22 minutes; paclitaxel, 25.5 minutes

Note 1

Mobile phases containing acetonitrile may be used in place of methanol, however, some adjustment in mobile phase gradient conditions may be desired.

Note 2

6(alpha)-Hydroxypaclitaxel and paclitaxel can be separated with most C18 columns. However, some adjustment in mobile phase gradient conditions 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

Cancer Res. (1994) 54, 4026; J. Med. Chem. (1994) 37, 706; Cancer Res. (1994) 54, 5543; J. Chromatog. B (1995) 674, 261.