CONCEPT LIFE SCIENCES

DELIVERING SCIENCE

DATA SHEET

MICROSOME & S9 STABILITY

Metabolic stability has a profound influence on therapeutic efficacy and toxicity. The majority of drugs are biotransformed by metabolic enzymes into more polar molecules that are readily excreted. Rapid metabolism lowers drug exposure at the therapeutic target. Microsomes are sub-cellular liver fractions containing phase I enzymes and are the most commonly used reagent for determining stability as most drugs undergo CYP mediated metabolism.

CUSTOMER PROVIDES

Compound identifier and molecular formula.
Test: 25µLof 10mM in DMSO or 0.5mg solid.

ENZYMES

Pooled liver microsomes or S9 are available in a range of species e.g. human, dog, rat, mouse & monkey.

TEST COMPOUND

Incubation concentration 1µM.

FORMAT

96-well plate, shaking incubator at 37°C.

PROTOCOL

Microsomes 1mg protein/mL. 350µL incubation volume. Microsomes are pre-incubated with NADPH cofactor solution at 37°C. Biotransformation is initiated by addition of test compound, and mixing. Standard time-points 0, 5, 10, 15, 25, 35 min (or client specific).

S9 1mg protein/mL. 350µL incubation volume. S9 are pre-incubated with NADPH and UDPGA cofactor solution at 37°C. Biotransformation is initiated by addition of test compound, and mixing. Standard time-points 0, 5, 10, 15, 25, 35 min (or client specific).

The final solvent concentrations in the incubation are 0.99% methanol and 0.01%DMSO.

At each specified time-point, a sample aliquot (25µL) is removed from the test incubation mixture and immediately combined into a cassette of up to 4 compounds, in 300µL ice cold methanol containing internal standard, and mixing to stop the reaction.

After the final time-point, the quenched samples are then centrifuged (4000rpm at 4°C for 30min) to precipitate the protein.

POSITIVE CONTROLS

Standard compounds Dextromethorphan, Diazepam, Midazolam and Phenacetin are used in each assay run.

QUANTITATION

The supernatants are analysed by LC-MS/MS using Concept Life sciences generic analytical methods to measure the test parent compound remaining at each time-point.

DATA ANALYSIS AND RESULTS

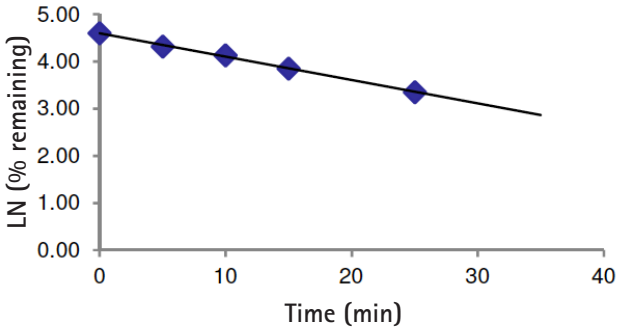
For each test compound injection:

$$\text{Response ratio} = \frac{\text{Test peak area}}{\text{Internal standard peak area}}$$

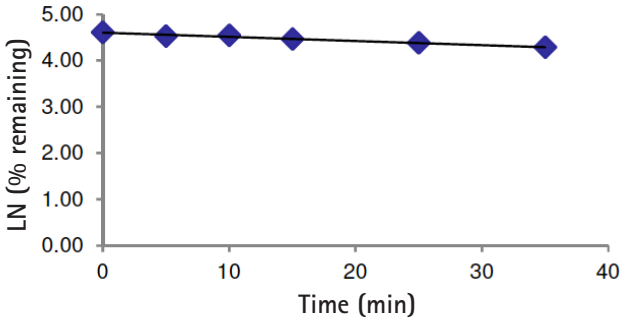
The natural log of the test compound response ratio is plotted versus time. The -ve slope of the semilog plot gives the elimination rate constant k (min^{-1}), from which intrinsic clearance Cl_{int} (µL/min/mg microsomal protein) and half-life $t_{1/2}$ (min) are calculated.

$$\text{E.g. } t_{1/2}(\text{min}) = \frac{\text{Ln2}}{k(min^{-1})}$$

DEXTROMETHORPHAN



PHENACETIN



CONCEPT HUMAN LIVER MICROSOMES