CONCEPT LIFE SCIENCES

DELIVERING SCIENCE

DATA SHEET

HEPATOCYTE 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. Hepatocytes are isolated whole liver cells and are the best in vitro system for modelling metabolism as they contain the full complement of drug metabolising enzymes with phase I & phase II co-ordinated activity.

CUSTOMER PROVIDES

Compound identifier and molecular formula.

Test: 25µLof 10mM in DMSO or 0.5mg solid.

ENZYMES

Pooled cryopreserved hepatocytes-phase I & phase II (Corning and IVT). Human and other species are available e.g. dog, rat, mouse.

TEST COMPOUND

Incubation concentration 1µM.

FORMAT

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

PROTOCOL

Hepatocytes 0.5 x 10^6cells/mL. 300µL incubation volume. Cell suspension is equilibrated to 37°C and biotransformation is initiated by addition of compound solution, and mixing. Standard time-points 0, 5,10, 20, 40, 60 min (or client specific).

The final solvent concentrations 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, Phenacetin and Naloxone (phase II) 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

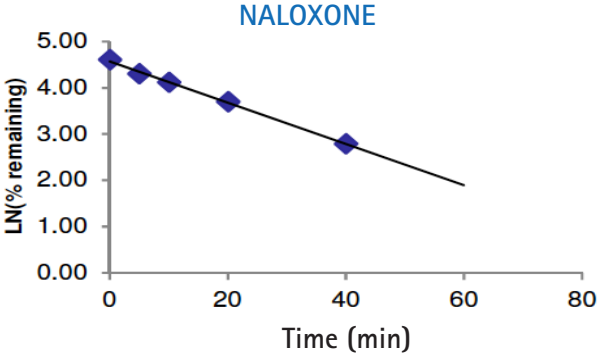
For each test compound injection:

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

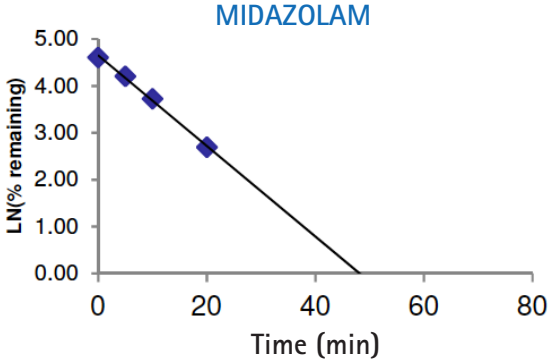
The natural log of the test compound response ratio is plotted versus time. The -ve slope of the semilog plot gives the elimination rate constant k (min^{-1}), from which intrinsic clearance Cl_{int} (µL/min/10^6 cells) and half-life $t_{1/2}$ (min) are calculated.

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

NALOXONE



MIDAZOLAM



CONCEPT MOUSE HEPATOCYTES