Contact us for more information
cls-admet.services@conceptlifesciences.com

CONCEPT LIFE SCIENCES

Concept Life Sciences:
Microsome & S9 Stability

Introduction

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 in vitro system for determining stability as most drugs undergo CYP mediated metabolism.

S9 are sub-cellular liver fractions containing Phase I and some Phase II enzymes present in the cytosolic fraction. S9 may be a preferred tool in early drug discovery to look at Phase I and Phase II mediated metabolism due to cost effectiveness when compared with hepatocytes.

Customer provides

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

Test compound

Incubation concentration 1µM.

Controls

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

Enzymes

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

Format

96-well plate, shaking incubator at 37ºC.

Protocol

Microsomes are diluted to an assay concentration of 1mg protein/mL and pre-incubated with NADPH cofactor for 10 minutes at 37^0C. Biotransformation is initiated by the addition of test compound and mixing. Standard time-points 0, 5, 10, 15,25, 35 minutes (or client specific). The final solvent concentrations are 0.99% methanol and 0.01% DMSO.

The S9 is diluted to an assay concentration of 1mg protein/mL in the presence of alamethicin (in MeOH) and $MgCl_2$, pre-incubated with NADPH and UDPGA cofactor for 10 minutes at 37^0C. Biotransformation is initiated by addition of test compound and mixing. Standard time-points 0, 5, 10, 15, 25, 35 minutes (or client specific).

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 centrifuged (4000rpm at 4^0C for 30 minutes) to precipitate the protein.

Quantitation

The supernatants are analyzed 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

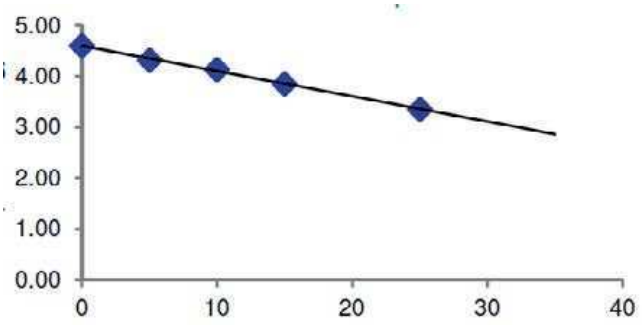
For each test compound injection:

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

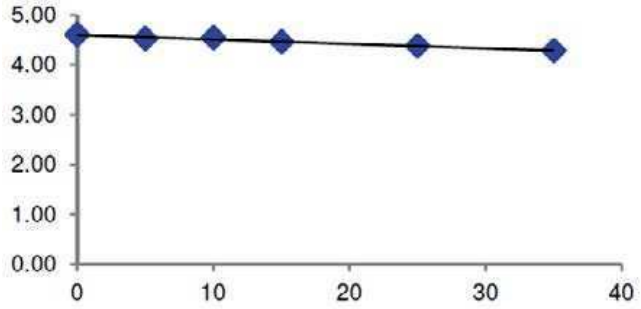
The natural log of the test compound response ratio is plotted versus time. The negative slope of the semi log plot gives the elimination rate constant k (min^{-1}), from which intrinsic clearance Cl_{int} (µL/min/mg microsomal protein) and half-life $t_{1/2}$ (min) are calculated.

$$t_{1/2}(\text{min}) = \frac{\ln 2}{k\,(\text{min}^{-1})}$$

Dextromethorphan



Phenacetin



Concept Human Liver Microsomes