



CONCEPT LIFE SCIENCES

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DATA SHEET

PLASMA STABILITY

Plasma stability plays an important role in drug discovery and development. Rapid metabolism lowers drug exposure at the therapeutic target. Furthermore unstable compounds cause difficulties for in vitro plasma protein binding studies, and in vivo PK studies since they continue to degrade after blood samples are taken from the animals. Plasma stability is also very useful for screening prodrugs where rapid conversion is desirable. Certain functional groups such as amides, esters or lactones make compounds favourable targets for plasma enzymes such as hydrolases and esterases.

Deliverable: % test compound remaining at each time-point.

CUSTOMER PROVIDES

Compound identifier and molecular formula.

Test: 25 μ L of 10mM in DMSO or 0.5mg solid.

PLASMA

Pooled heparinised available in a range of species e.g. human, dog, rat, mouse and monkey.

TEST COMPOUND

Incubation concentration 1 μ M.

FORMAT

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

PROTOCOL

Plasma is warmed to 37°C for 10 min, mixed and centrifuged to pellet any aggregated protein. The clear supernatant is aliquoted into the assay plate. Plasma is equilibrated

to 37°C and biotransformation is initiated by addition of compound solution, and mixing. 300 μ L final incubation volume. Standard time-points 0, 5, 15, 30, 60, 120 min (or client specific).

The final solvent concentrations are 0.99% acetonitrile and 0.01%DMSO.

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

After the final time-point, the quenched samples are stored at -20°C for a minimum of 4hr, then centrifuged (4000rpm at 4°C for 30min) to precipitate the protein.

POSITIVE CONTROLS

Two standard compounds benfluorex and eucatropine are used in each assay run.





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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

$$\text{For each test compound injection, response ratio} = \frac{\text{Test Peak Area}}{\text{Internal Standard Peak Area in Test}}$$

The response ratio is converted to % test compound remaining. The % test compound remaining is plotted versus time.

