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Metabolic stability assay in rat or dog microsomes

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We use this protocol and it's working

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Abstract

Metabolic stability, in the context of liver microsomes, refers to how quickly a drug candidate is metabolized (broken down) by the liver's enzyme system, specifically within the microsomes. This is measured by incubating a drug with microsomes and determining how much of the original drug remains after a set time. Microsomes are a fraction of liver cells enriched with enzymes, particularly cytochrome P450 (CYP) enzymes, which are responsible for metabolizing many drugs.

Safety warnings

- ⚠ Always wear appropriate PPE for this protocol
Refer to Material Safety Data Sheets for additional safety and handling information.

Summary

- 1 The metabolic stability in microsomes is performed in 96-well plate format
This protocol can be used with the following microsomes:
 - Rat
 - Dog

Sample Preparation

- 2 The compounds are incubated in 1 μ M concentration in a shaking incubator at 37°C
- 3 The relevant microsomes are diluted to a concentration of 1mg /mL and equilibrated at 37°C for 10 minutes in the presence of NADPH cofactor

Assay

- 4 Biotransformation is initiated by addition of compound and mixing
- 5 Standard time-points are used as 0, 5, 10, 15, 25, 35 min, and the final solvent concentrations are 0.99 % methanol and 0.01 % DMSO
- 6 At each specified time-point, a sample aliquot (25 μ L) is removed from the test incubation mixture and combined into a cassette of up to 4 compounds, in 300 μ L ice cold methanol containing internal standards (Dextromethorphan, Diazepam, Midazolam and Phenacetin), and mixing to stop the reaction
- 6.1 The quenched samples are then centrifuged to precipitate the protein.

Analytical

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

Data Analysis



- 8 Clearance and half-life are calculated from the elimination rate constant derived from plotting response ratio versus time

Protocol references

https://cdn.prod.website-files.com/659db4dde7f13f395f49157c/65f05dc5d8c335fad59bedfd_Concept%20Life%20Sciences_LE_MICROSO ME_AND_S9_STABILITY%202_.pdf

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