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Metabolic stability assay in human, rat, dog or mouse hepatocytes

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We use this protocol and it's working

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Disclaimer

Abstract

Metabolic stability, measured in hepatocytes, refers to the rate at which a drug compound is metabolized or broken down by liver cells. This is a critical aspect of drug discovery and development, as it helps determine how long a drug will remain in the body and at what concentration it will exert its therapeutic effect. In drug development, understanding metabolic stability is crucial for identifying compounds that are stable enough to reach their target and for optimizing chemical structures to improve metabolic stability.

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 the hepatocytes is performed in 96-well plate format
This protocol can be used with the following hepatocytes:
- Human
- Rat
- Mouse
- Dog

Sample Preparation

2 The compounds are incubated in 1 uM concentration in a shaking incubator at 37ºC

3 The relevant hepatocytes are diluted to a concentration of 0.5×10^6 cells /mL and equilibrated at 37ºC for 10 minutes

Assay

4 Biotransformation is initiated by addition of compound and mixing

5 Standard time-points are used as 0, 5, 10, 20, 40, 60 min, and the final solvent concentrations are 0.99 % methanol and 0.01 % DMSO

6 At each specified time-point, a sample aliquot (25µL) is removed from the test incubation mixture and combined into a cassette of up to 4 compounds, in 300µL ice cold methanol containing internal standards (Dextromethorphan, Diazepam, Midazolam, Phenacetin and Naloxone), 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

9 For each test compound injection,

Response rate = Test peak area / 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 Clint (µL/min/106 cells) and half-life t1/2 (min) are calculated.

E.g. t1/2 (min) = Ln2 / k(min-1)

Protocol references

https://cdn.prod.website-files.com/659db4dde7f13f395f49157c/65f05da357b031fab6bc2e50_Concept%20Life%20Sciences_LE_HEPATOCYTE_STABILITY%20_2_.pdf

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