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In-vitro CYP inhibition pooled

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Abstract

This assay is crucial for understanding a compound's inhibitory profile across major drug-metabolizing enzymes, providing essential data for predicting clinical drug-drug interactions and supporting regulatory submissions in pharmaceutical development.
An in vitro CYP inhibition assay evaluates the potential of a test compound to inhibit multiple cytochrome P450 enzymes simultaneously using a cocktail approach. This pooled assay employs a mixture of substrates specific to different CYP isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6 & CYP3A4) combined with a pooled enzyme source, such as human liver microsomes.
The cocktail method offers several advantages over individual isoform testing, including:

- Reduced material consumption - Single test compound aliquot tests multiple CYP enzymes
- Higher throughput screening - Multiple CYP interactions assessed simultaneously
- Early drug development efficiency - Rapid identification of potential drug-drug interaction liabilities

Safety warnings

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

Summary

1 The pooled CYP inhibition is performed in 96-well plate format, the compounds are incubated at 0, 0.15, 0.5, 1.5, 5, 15 & 50µM concentration in a shaking incubator at 37ºC

Sample Preparation

2 CYPs are pooled in a ratio such that biotransformation of each probe substrate is specific for the particular CYP450

3 Substrates (at their Km) are also pooled within the same solution to create an enzyme-substrate stock and test compound is added to appropriate wells

Assay

4 The final solvent concentration is 1.0% DMSO

5 After equilibration to 37ºC, addition of NADPH and mixing initiates substrate biotransformation

6 Each concentration of test compound is assayed against five CYPs in the same well simultaneously.

6.1 The incubation is stopped at t = 10min by removal of the plate from the shaking incubator, followed by the addition of 200µL of ice cold methanol containing internal standard, and mixing.

7 The quenched samples are then centrifuged to precipitate the protein.

Analytical

8 The supernatants are analyzed by LC-MS/MS using generic analytical methods to measure metabolite formation.

Data Analysis

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

10 IC_{50} (µM) of test compound against CYP1A2, CYP2C9, CYP2C19, CYP2D6 & CYP3A4 are calculated by plotting 6-point inhibition curves of the metabolite formation response.

Protocol references

https://cdn.prod.website-files.com/659db4dde7f13f395f49157c/65be2ac7323eab7e4d4b0d62_Concept%20Life%20Sciences_LE_CYP450_ISOFORM.pdf

Acknowledgements

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