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In-vitro Mouse or Human Blood stability assay

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Nick Lynch^{1,2}

¹Curlew Research; ²ASAP Discovery Consortium

ASAP Discovery



Nick Lynch

Curlew Research, ASAP Discovery Consortium

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Protocol status: Working

We use this protocol and it's working

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Abstract

In vitro blood stability is a key aspect of DMPK (Drug Metabolism and Pharmacokinetics) research, focusing on how a drug candidate behaves within the blood in a controlled laboratory environment. This involves evaluating the compound's stability, its rate of degradation, and its potential for interaction with other blood components.

Safety warnings

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

Summary

- 1 The in-vitro mouse blood stability incubation assay is run in 96-well plates on a Thermomixer set to 39°C and 700 rpm
This protocol can be used with the following blood samples:
 - Human (HBS)
 - Mouse (MBS)

Sample Preparation

- 2 Fresh blood is aliquoted (297 µL) into relevant plate wells
- 3 Test compounds are diluted to 100 µM in methanol, and 3 µL is added to initiate the reaction

Assay

- 4 Quenching occurs at specified time points (0–120 minutes) by transferring 25 µL of the incubate into wells pre-filled with ice-cold acetonitrile containing internal standard. This is repeated column-by-column to maintain precise timing
- 5 After incubation, plates are sealed and stored overnight at 4°C.
- 6 Samples are centrifuged, and 150 µL of supernatant is transferred to a new plate, diluted with 30% methanol in water to a final volume of 300 µL
 - 6.1 Method (MS) blanks are also prepared
 - 6.2 Samples are injected onto LC-MSMS and the % remaining of the test compound at each time point calculated against T0

Data Analysis

- 7 The percent remaining of the test compound against time is calculated



For each test compound injection,

response ratio = Test peak area / Internal standard peak area in test

The response ratio is converted to % test compound remaining.

The % test compound remaining is plotted versus time.

Protocol references

https://cdn.prod.website-files.com/659db4dde7f13f395f49157c/65f05d5b0ae1671d5fff063_Concept%20Life%20Sciences_LE_PLASMA_STABILITY%202_.pdf

Acknowledgements

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