Cultured Hepatocyte Xenobiotic Metabolism Assay

Introduction

Freshly isolated hepatocytes represent a living biological system in which coupled phase I and II chemical metabolism can be evaluated.[1]

Purpose

This assay is designed to study what metabolites, if any, are generated following the incubation of hepatocytes with the test article. This assay does not address how the metabolites are formed, or which specific enzymes are involved.

Principle of the Procedure

Cultured hepatocytes are incubated with xenobiotics to allow the generation of metabolites.

Materials and Reagents*

Item	Manufacturer	Name/Catalog/Model #
Biosafety cabinet	NuAire, Inc.	NU425FM600
CO_2 water-jacketed incubator	Forma Scientific	3110
Plastic multi-well plates	Costar	
Plated hepatocytes	BioreclamationIVT	
Pump	GAST	DPA104AA
Solvents/Solubilizers		
Acetonitrile (ACN)	Aldrich	27,071-7
Dimethyl sulphoxide (DMSO)	Sigma Chemical Co.	D-2650
Methanol	Sigma Chemical Co.	270474
Buffers		
*InVitro*GRO™ HI Medium	BioreclamationIVT	Z90009

*Items listed in this Materials section are for convenience; suitable materials and equipment from other manufacturers may be substituted as appropriate. Contact information for vendors used by BioreclamationIVT are listed in the Notes section at the end of this document.

Procedure

1. Prepare test article in BioreclamationIVT *InVitro*GRO HI Medium. To solubilize lipophilic test articles, use ACN as a solvent (and only as necessary). The final concentration of the solvent should be kept at or below 1%.

2. Prepare a negative control sample. Use BioreclamationIVT *InVitro*GRO HI Medium containing any solvent used to dissolve the test article as a negative control. Each plate should contain a negative control.

3. Once the test article has been prepared, transfer the plate from incubator to the biosafety cabinet, aspirate the BioreclamationIVT *InVitro*GRO HI Medium, and replace it with an equal volume of each test article dissolved in incubation media. Appropriate volumes are
   - 100 µL per well for 96-well plates.
   - 500 µL per well for 24-well plates.
   - 1.0 mL per well for 12-well plates.
   - 2.5 mL per well for 6-well plates.

4. Replace the lid on the plate. Return the plate to the 5% CO_2, 37 °C incubator for an appropriate length of time (1, 4, and 16–24 hours is typical).

5. At the end of the incubation period, harvest the media from each well into a suitable storage vessel. Store at a temperature (typically between –20 and –70 °C) appropriate for test article stability. As the cells may retain a significant portion of parent compound and/or metabolites, the remaining plated cells should be stored for later analysis.

6. Analyze media samples by HPLC and/or LC/MS for parent and possible metabolites. Hepatocytes remaining in the plates after incubation may be extracted with methanol or another appropriate solvent for analysis. Compare the test article chromatograms against the HPLC or LC/MS profile of the negative control.

References

1. Li, A. P. Primary hepatocyte cultures as an in vitro experimental model for the evaluation of pharmacokinetic drug-drug interactions. *Adv. Pharmacol. Series* **1997**, *43*, 103–130.

Cautions

Treat all products containing human- and monkey-derived materials as potentially infectious, as no known test method can offer assurance that products derived from human or monkey tissues will not transmit infectious agents.

All products are for research use only. Do not use in animals or humans. These products have not been approved for any diagnostic or clinical procedures.