CYP450 Inhibition

Overview

- The main drug-metabolizing system in mammals is cytochrome P450 (P450), a family of microsomal enzymes occurring predominantly in the liver.
- P450 enzymes are responsible for the bulk of the metabolism of known drugs in humans.
- When a drug that is metabolized by a particular P450 is co administered with an inhibitor of that same enzyme, changes in pharmaco-kinetics occur, and can give rise to adverse effects.
- Inhibition of these enzymes by co-administered drugs has led to the withdrawal of several drugs from the market.
- It is therefore important to predict and prevent the occurrence of clearance changes due to metabolic inhibition.
- During the drug discovery process, assessing drug safety in the early development stages is crucial in identifying and eliminating compounds that might later exhibit a potential for undesirable DDIs.

Protocols

The following 16 P450 inhibition assays are currently offered at MEDINA.

Compounds can be tested at single concentrations (typically 10 µM) or multiple concentrations (typically 0.03, 0.1, 0.3, 1, 3, 10, 30, and 100 µM) for IC50 determinations (custom dilution patterns and final assay concentrations are available):

CYP	Substrate	Microsomes	Detection
CYP1A2	CEC	Recombinant	Fluorometry
CYP2C8	DBF	Recombinant	Fluorometry
CYP2C9	MFC	Recombinant	Fluorometry
CYP2C19	CEC	Recombinant	Fluorometry
CYP2D6	AMMC	Recombinant	Fluorometry
CYP3A4	BFC	Recombinant	Fluorometry
CYP1A2	phenacetin	Liver	LC-MS/MS
CYP2C8	paclitaxel	Liver	LC-MS/MS
CYP2C9	diclofenac	Liver	LC-MS/MS
CYP2C19	(S)-mephenytoin	Liver	LC-MS/MS
CYP2D6	dextromethorphan	Liver	LC-MS/MS
CYP3A4	midazolam	Liver	LC-MS/MS
CYP3A4*	testosterone	Liver	LC-MS/MS

* Also available in time dependent inhibition

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info@medinaandalucia.es

MEDINA offers a range of assays to determine the potential for drug-drug interactions:

High-throughput fluorescent CYP inhibition

Fluorometric assays based on substrates metabolism to highly fluorescent products represent the most common approach for testing compounds as CYP inhibitors in early drug discovery.

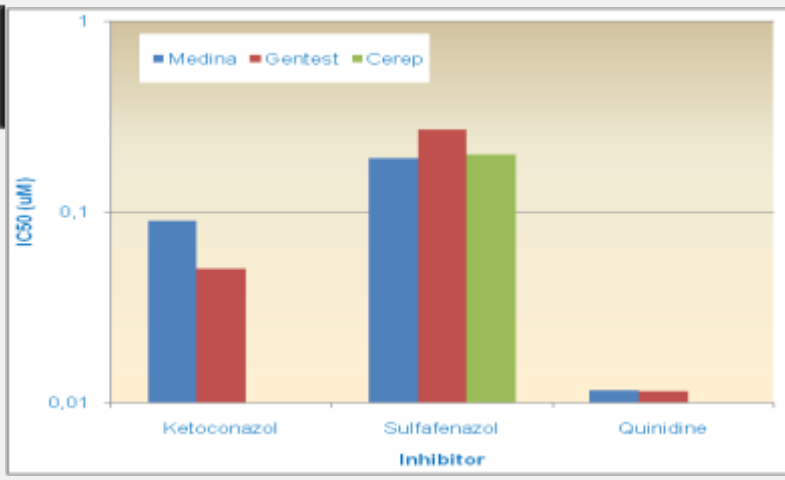

Figure 1. Interlab comparison for control Inhibitor on fluorescence based assays

LC/MS CYP inhibition

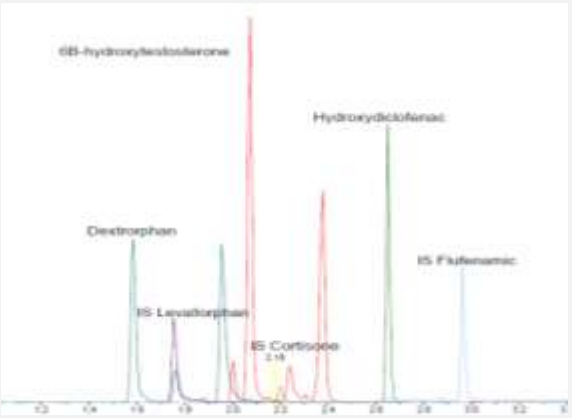

Figure 2. Probe substrates and IS LC/MS/MS chromatogram

A more definitive and confirmative investigation of CYP inhibition using traditional probe substrates

Ketoconazol CYP3A4 IC50

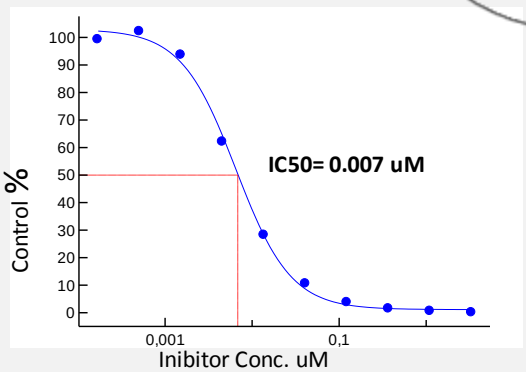

Figure 3. IC50 Calculation for Ketoconazol on CYP3A4 radiometric assay.

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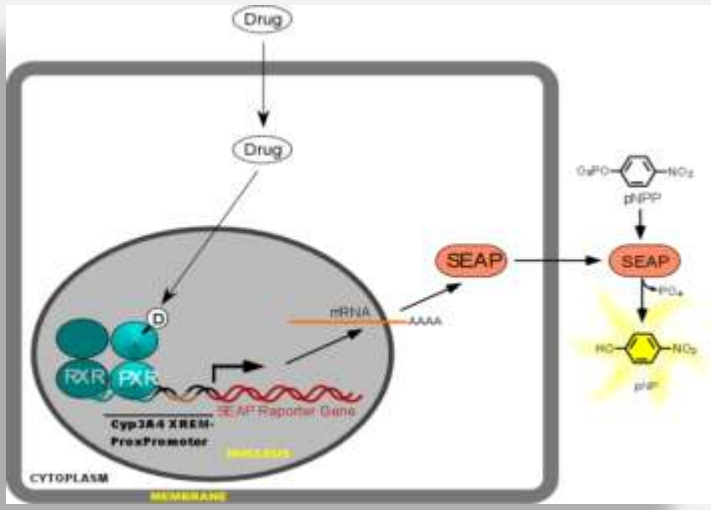
CYP450 Induction

Overview

- Why worry about CYP 3A4 inducers?
  - 50-60% of all drugs are metabolized by CYP 3A4.
  - Drug/drug interactions
    - Rifampin reduces plasma levels of oral contraceptives (Back et al. 1980)
  - Induction of own metabolism
    - (Ex. Rifampicin etc.)
- CYP3A4 induction assay principle
  - The nuclear receptor, PXR (pregane X receptor), is required for induction of CYP3A4 (Xie et al, Nature 406:435, 2000).
  - The CYP3A4 gene contains 4 PXR binding sites (Goodwin et al, Mol. Pharm. 56:1329, 1999).



Protocols

Day 1

- Transient transfected Hep G2 cells in T175 flasks with hPXR + Cyp3A4-SEAP.

Day 2 Morning

- Trypsinize cells and replate in 96-well plates, ~5-8 x10^4 cells/well.

Day 2 Afternoon

- Add drugs to cells. Ten point DRCs in duplicate. Incubate 48 hrs.

Day 4

- Add SEAP buffer, read 410 nm (3 readings).
- Analyze data [SEAP activity rate = Δ 410nm/min].

Positive Control

- Rifampicin

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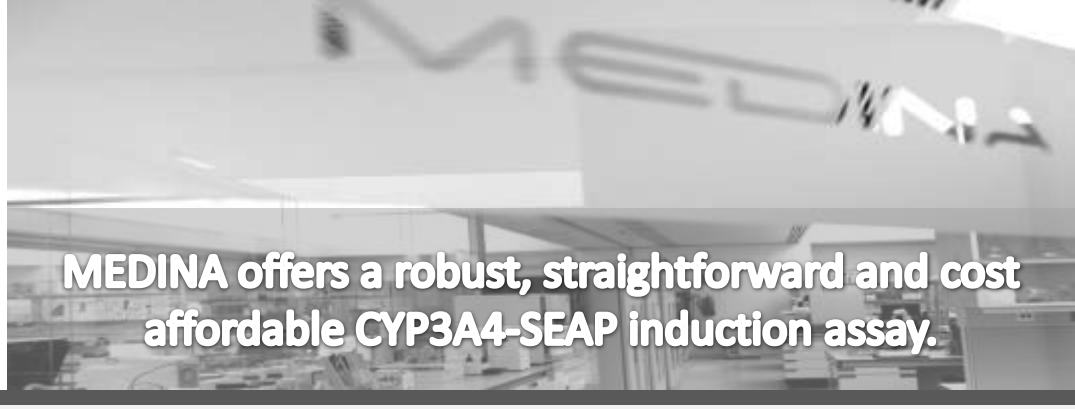

MEDINA offers a robust, straightforward and cost affordable CYP3A4-SEAP induction assay.

Assay validation

The assay was validated by comparing data generated in the primary hepatocyte CYP3A4 induction assay with the CYP3A4-SEAP assay.

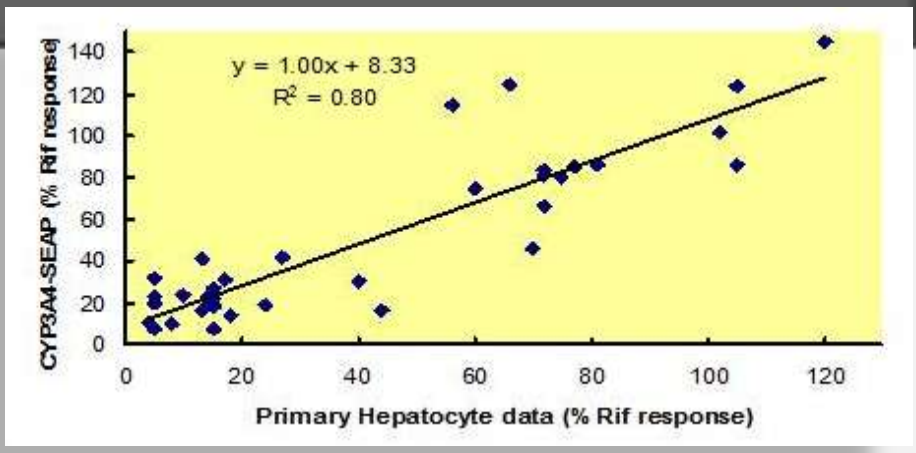

Figure 1. Intercomparison of data for 36 compounds tested in the primary hepatocyte assay and the PXR-CYP3A4 SEAP assay.

MEDINA also offers the following drug metabolism services:

In Vitro cytochrome P450 reaction phenotyping (Enzyme identification).

Reaction phenotyping (enzyme identification) studies are conducted to identify the specific enzymes responsible for the metabolism of a drug. Identification of cytochrome CYP450 enzymes *in vitro* can prove useful in predicting the potential for drug interactions, polymorphic impact on drug disposition, and formation of toxic or active metabolites.

Individual recombinant human CYP450 isoforms are valuable tools for evaluating the intrinsic ability of each individual isoform to metabolize a drug candidate.

Metabolic Stability Liver Microsomes

Metabolic stability is defined as the percentage of parent compound lost over time in the presence of a metabolically active test system and represents an important property of drug candidates since it affects parameters such as clearance, half-life and oral bioavailability. Therefore metabolic stability should be evaluated early in the drug discovery and development process.

In vitro methods using liver microsomes provide valuable test system of choice to study Metabolic stability.

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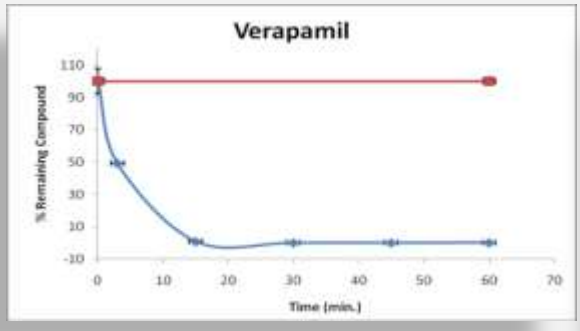
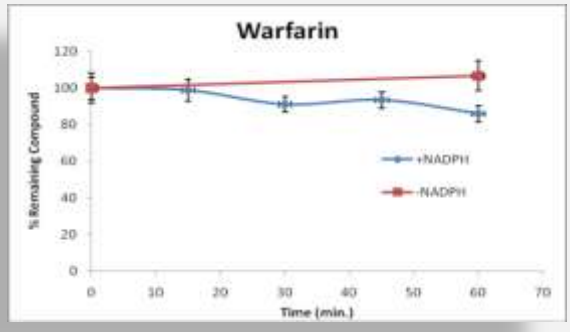
Metabolic Stability

Overview

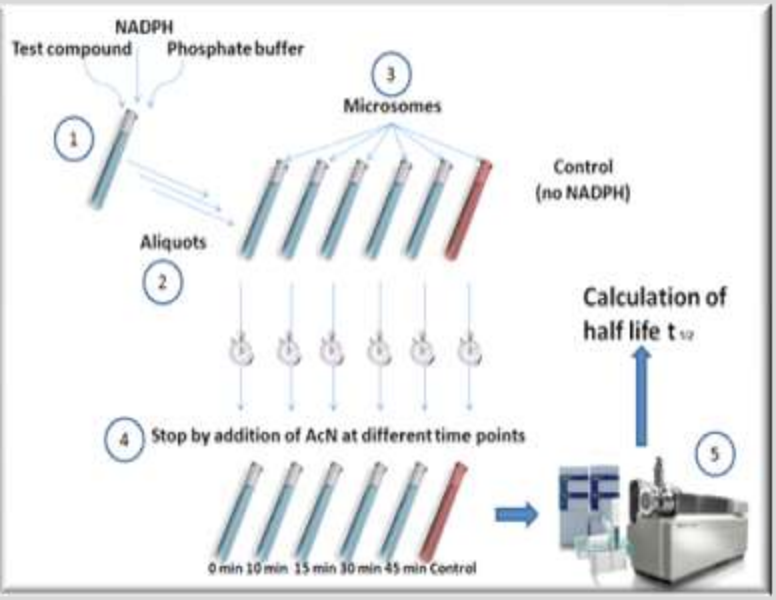
○ Extensive metabolism is responsible for high clearance and 1st-pass effect, reducing the half-life and bioavailability of potential drugs, compromising the possibility for investigated molecules to elicit their activity at the targeted organ.

○ In vitro metabolic stability is used to predict in vivo hepatic clearance due to metabolism and is a well-established and potent method for screening a high number of compounds in a cost-effective and simple manner.





Protocols



Test conditions:
Test Compound Concentration 1 µM (different cons. available)
Microsome Concentration 1 mg/ml (different con. available)
Time Points 0, 5, 15, 30, 45 minutes
Cofactor NADPH
Final DMSO Concentration 0.02 %
Compound Requirements 50 µL of 10 mM solution
Controls
Minus cofactor (45 min only);
Positive control compounds with known activity
Analysis method
LC-MS/MS
Data Delivery
Intrinsic clearance
Half life

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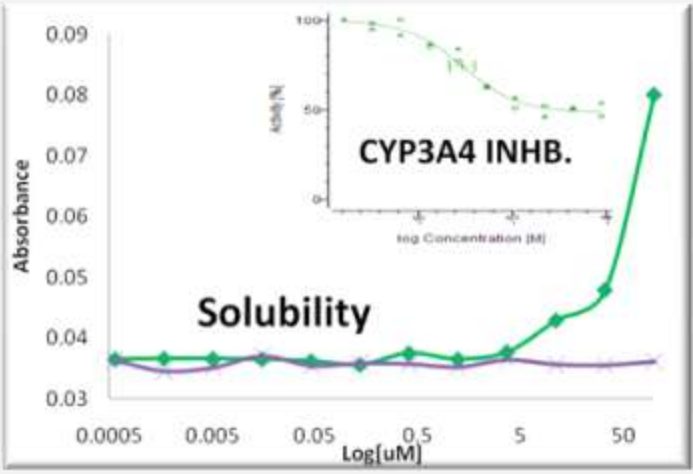
Metabolic Stability

Overview

- Compounds with poor aqueous solubility can precipitate during in vitro assays and result in lower concentration than expected in the assay.

- In an activity-screening assay, this can involve missing active compounds with low solubility.

- In a CYP450 inhibition assay, compounds with poor solubility can show artificially low inhibition, over looking compounds with potential drug–drug interaction toxicities.



Early solubility screening is key to ensure successful drug discovery.

Protocols

Final test compound concentration 1 µM, 3 µM, 10 µM, 30 µM and 100 µM
Buffer 0.1 M phosphate buffered saline pH7.4
(alternatives available on request)
Final DMSO concentration 1 %
Incubation Time 2 hr
Incubation Temperature 37°C
Analysis method Absorbance at 620 nm
Data Delivery Estimated solubility range (lower and upper bound and calculated mid-range in µM).

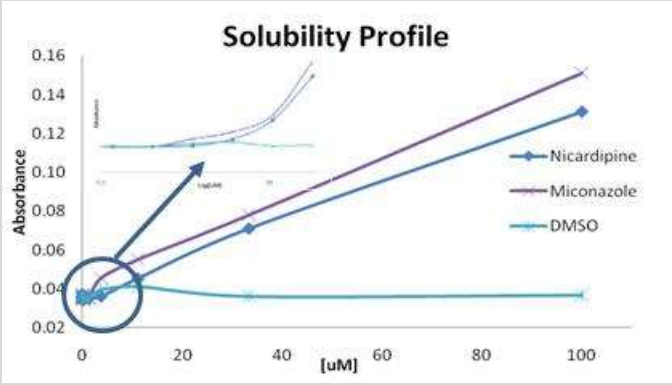


Table 1 Measurement of the Kinetic Solubility of Marketed Drugs

Compound	MEDINA Solubility Range	Solubility Category[1]
Verapamil	>100 uM	Acceptable (KS > 50 uM)
Diclophenac	>100 uM	
Nifedipine	11-33 uM	Marginal (KS = 10–50 uM)
Haloperidol	4-11 uM	
Nicardipine	4-11 uM	
Bromoergocriptine	1-4 uM	Unacceptable (KS < 10 uM)
Clotrimazole	1-4 uM	
Miconazole	1-4 uM	

(1) Rogge MC, Taft DR, editors. 2010. Preclinical Drug Development. second ed: Informa Healthcare USA, Inc.

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info@medinaandalucia.es

Hepatocity Stability

Overview

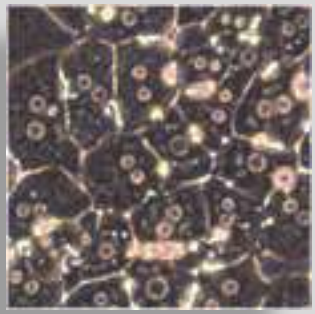
○ Cryopreserved hepatocytes can be used to perform experiments where an intact cellular system is required. Intact, viable hepatocytes contain the major hepatic drug-metabolizing enzymes, (including reticular systems, citosolic and mitochondrial enzymes) as well as the co-factors required to study the four categories of biotransformation.

○ Hepatocytes stability assay can be used to profile for metabolites formed by both phase

○ I and phase II enzymes. Options include an analysis to identify whether a metabolite is formed, or a cross species comparison to identify potential differences in metabolism which could in turn help to interpret pharmacology and toxicity data.

Protocols

Cells Criopreserved hepatocytes
Species Human, rat, mouse, dog, primate, minipig, rabbit, guinea pig (other species available)
Test Compound Concentration 3 µM (different concentrations available)
Time Points 0, 5, 10, 20, 40, 60 minutes
Final DMSO Concentration 0.25 %
Compound Requirements 50 µL of 10 mM solution
Controls
Known substrates which undergo either phase I or phase II metabolism
Heat-inactivated hepatocyte control incubation for each compound
Vehicle control incubation
Analysis method
LC-MS/MS
Data Delivery
•Intrinsic clearance
Half life
•Ion chromatogram of parent and propose metabolites
•Name of propose metabolites and where possible molecular formulae
•MSMS spectra of parent and proposed metabolites
•Details of the product ion fragment of parents and any potential of metabolites
•Confirmation of metabolites identified by LCMS scan



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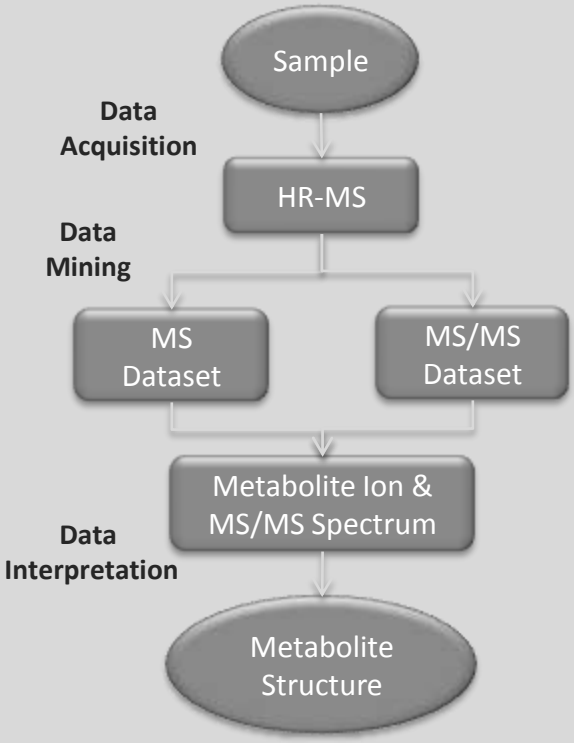
Metabolite Profiling and Identification by high-resolution mass spectrometry

Overview

- The development of high-resolution (HR) MS instrumentation with improved accuracy and stability, along with new data processing techniques, has improved the quality and productivity of metabolite identification processes.
- HR-Spectrometer Have the potential to simultaneously provide both qualitative and quantitative information on multiple analytes including metabolites.
- FUNDACIÓN MEDINA has recently acquired an AB SCIEX TripleTOF® 5600 System which integrates comprehensive qualitative exploration, rapid profiling, and high-resolution quantitation workflows on a single platform.

General Scheme



Deliverables:

- Ion Chromatogram of parent and proposed metabolites
- Name of proposed metabolites , molecular formula and when possible metabolite structure.
- Table containing metabolite HR mass, mass error, metabolite score, absolute areas and retention time.

FUNDACIÓN MEDINA offers these studies in different biological matrix including liver microsomes , hepatocites, expressed enzymes and plasma samples

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