DISCOVERY SERVICES

DRUG DISCOVERY

Cytochrome P-450 Subtype Inhibition Studies

Background

Many drug-drug interactions are metabolism-based and of these, most involve Cytochrome P-450 (CYP) enzymes. Eleven xenobiotic metabolizing CYPs are expressed in a typical human liver (CYP1A2, CYP2A6, CYP2B6, CYP2C8/9/18/19, CYP2D6, CYP2E1 and CYP 3A4/5). Of these, six principal enzymes (CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) appear to be the most commonly responsible for the metabolism of most drugs and the associated drug-drug interactions. This is primarily due to the preference of these enzymes to bind and/or metabolize chemical structures commonly found in drugs, and due to the mass abundance of some of these enzymes in human liver.

Key Features of the Assay

- available for the following nine enzymes: CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4
- rapid throughput fluorescence-based assay

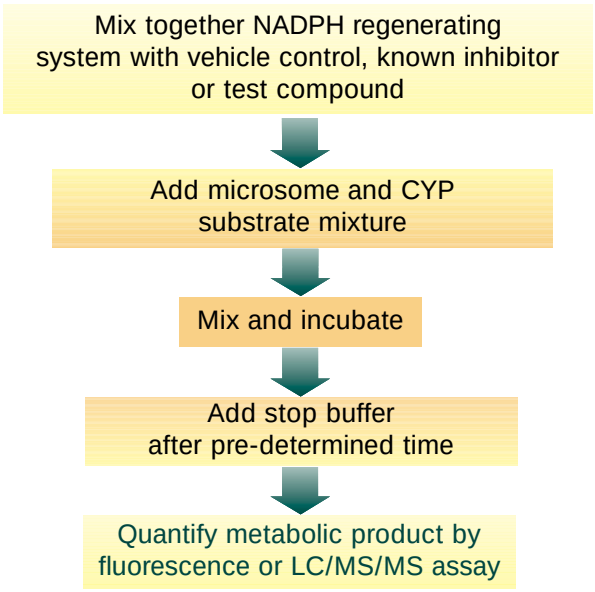
Assay Applications

- identifies which metabolizing enzyme a drug is interacting with
- predicts the potential for a new drug to interact with co-administered drugs

Assay Principle

The assays use microsomes prepared from insect cells, each expressing an individual CYP subtype (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9*1, CYP2C19, CYP2D6*1, CYP2E1 or CYP3A4) expressed from the corresponding human CYP cDNA using a baculovirus expression vector. The assays monitor, *via* fluorescence detection, the formation of a fluorescent metabolite following incubation of the microsomes with a specific CYP substrate. Because inhibition constants are substrate selective for CYP3A4, at least two substrates are commonly examined for this enzyme. This rapid throughput assay does not distinguish between a substrate and inhibitor. Further studies can be performed to characterize substrates and inhibitors.

Assay Protocol



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Typical Results

CYP Subtype	Substrate	Inhibitor	Inhibitor IC_{50} (µM) $\bar{x}$ ± SD (N)
CYP1A2	CEC	Furafylline	2.3 ± 0.4 (3)
CYP2A6	Coumarin	Tranycypromine	0.64 ± 0.18 (4)
CYP2B6	EFC	Tranycypromine	16.6 ± 0.8 (4)
CYP2C8	DBF	Quercetin	0.9 ± 0.08 (4)
CYP2C9	7-MFC	Sulfaphenazole	0.26 ± 0.1 (5)
CYP2C19	CEC	Tranylcypromine	4.37 ± 0.9 (4)
CYP2D6	AMMC	Quinidine	0.003 ± 0.0006 (3)
CYP2E1	7-MFC	DDTC	8.4 ± 2.8 (7)
CYP3A4	BFC	Ketoconazole	0.011 ± 0.006 (4)
	BQ		0.2 ± 0.08 (4)

Typical IC_{50} values obtained for inhibitors of the various CYP subtypes. These values correspond to those reported in the literature.

CEC: 7-Ethoxy-3-cyanocoumarin
EFC: 7-Ethoxy-4-trifluoromethylcoumarin
DBF: Dibenzyfluorescein
DDTC: Diethyldithiocarbamic acid
7-MFC: 7-Methoxy-4-tifluoromethylcoumarin
AMMC: 3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methylcoumarin
BFC: 7-Benzyloxyquinoline
BQ: Benzyloxyquinoline

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