BIOPREDIC INTERNATIONAL

SILENSOMES®

SILENSOMES™: Step into the future with fm prediction! Replace RAF assays with a new in vitro tool for phenotyping

Silensomes™ and accurate fm prediction

Prediction of drug-drug interactions is required in the development of all new drug candidates. In vitro identification and measurement of the contribution of the major cytochrome P450 (CYP) enzymes involved in the metabolism of a drug candidate, also called "CYP phenotyping", allow the prediction of the impact of co-administered drugs on their pharmacokinetics.

Until now, these studies are carried out using three common approaches: correlation analysis, antibody or chemical inhibition and metabolism by recombinant human enzymes. Each has its advantages and disadvantages; therefore; a combination of approaches is recommended (FDA/EMA) to reliably identify the CYP(s) involved in the metabolism of a compound.

To address the disadvantages of the current methodologies, Biopredic International has co-developed with Servier Laboratories a patented new in vitro drug development tool, called Silensomes™

ADVANTAGES OF SILENSOMES™:

A simple solution for phenotyping
Accurate prediction of in vivo fm
Comply with FDA DDI guidelines

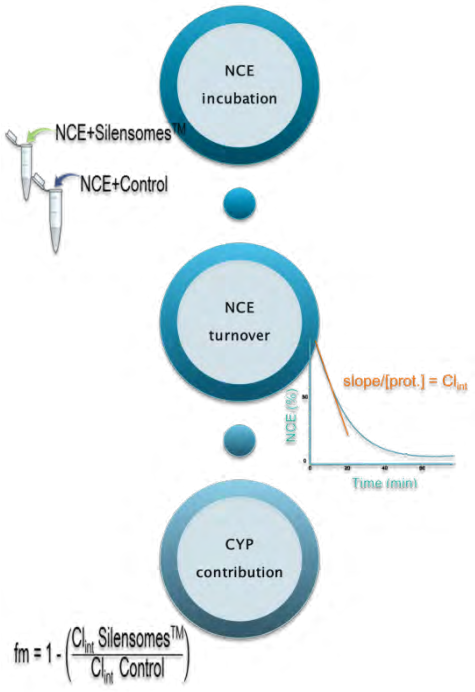
What they are...

Silensomes™ are validated human pooled liver microsomes (HLMs) in which a single CYP has been chemically and irreversibly inactivated using mechanism based inhibitors (MBI).

There are 9 CYP-Silensomes™ each with a single inactivated CYP activity (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4). Inhibition is highly specific and extensive (>80 %), with only minor impact (<20%) on the other CYP activities.

A simple assay!

Incubate the test compound with CYP-silenced and homologous control microsomes and compare intrinsic clearances (Clint).



Principle for phenotyping and fm assay with CYP-Silensomes™

Patent N°PCT/EP2014/076748 & US 14/561,817 – Publication « submitted in progress »

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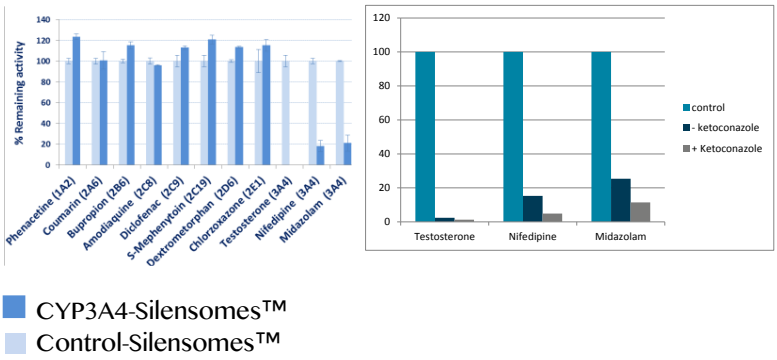
A case study with CYP3A4-Silensomes™

CYP3A4 activity is specifically and extensively inhibited in CYP3A4-Silensomes™

CYP3A4-Silensomes™ and its homologous control were incubated with different CYP-specific substrates. Results showed:

- **CYP3A4 mediated metabolism of testosterone, a pure CYP3A4 substrate, was totally inhibited.**
- More than 80% of CYP3A4-mediated metabolism of nifedipine and midazolam was inhibited. Residual metabolism of these substrates was inhibited by ketoconazole, **revealing the CYP3A5 contribution.**
- **There was no impact on the other CYP activities tested.**



The CYP3A4-Silensomes™ directly predict the CYP contribution to drug metabolism

CYP3A4-Silensomes™ and its control were incubated with drugs known to be metabolized by CYP3A4 with varying contributions. Incubation conditions were optimized to allow measurement of their intrinsic clearance. The Clint ratio represents CYP3A4 contribution:

$fm = 1-(Cl_{int}$ Silensomes™ / Cl_{int} Control)

The fm determined by the rhCYP model normalized by the relative activity factor (RAF) varies depending on the substrate used.

By contrast, the CYP3A4 contribution to all tested drugs using CYP3A4-Silensomes™ correlated well with reported in vivo data.

Estimated % CYP3A4 contribution

Drug	Silensomes™	Human recombinant models *(internal data)*		Literature data
		Using RAF Nifedipine	Using RAF Testosterone	in vivo
Mirtazapine	**24**	5	30	**33** [a]
Loperamide	**53**	57	90	**62** [b]
Omeprazole	**37**	17	42	**27** [c]
Bortezomib	**73**	37	55	-
Midazolam	**88**	76	95	**86** [d]
Nifedipine	**77**	54	90	**74** [e]

(a)Mirtazapine FDA Notice; (b)Tayrouz et al., (2001); (c)Bottiger et al., (1997); (d)Tsunoda et al., (1999);(e) Yamazaki et al., (1996)

CYP3A4-Silensomes™ are the most appropriate tool to predict CYP3A4 contribution to drug metabolism.

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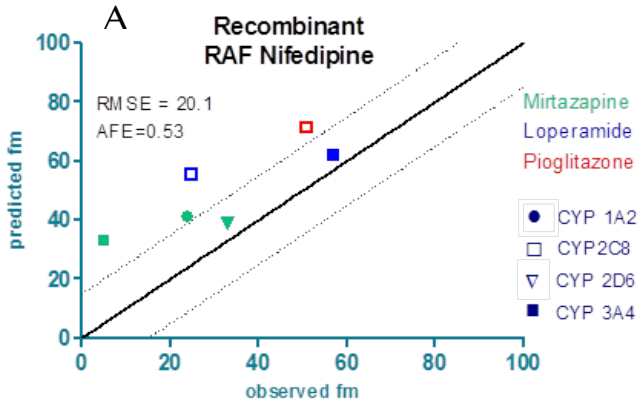
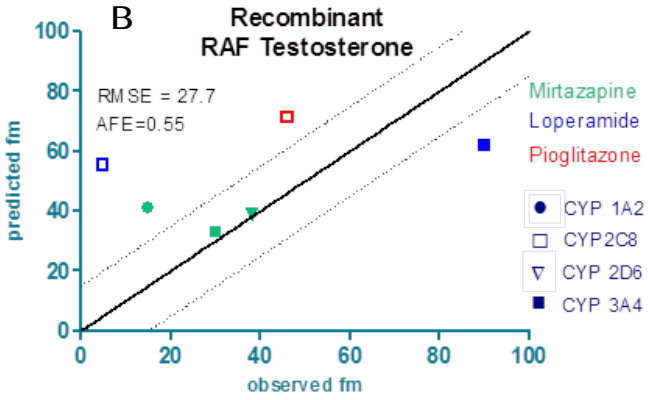
CYP3A4-Silensomes™ - an accurate fm prediction - the RAF is history!

The fm for 4 drugs calculated using RAF and Recombinant CYP3A4, results in different contributions depending on the substrate used i.e. nifedipine or testosterone. Moreover, the fm does not correlate well with in vivo data (Fig A & B).

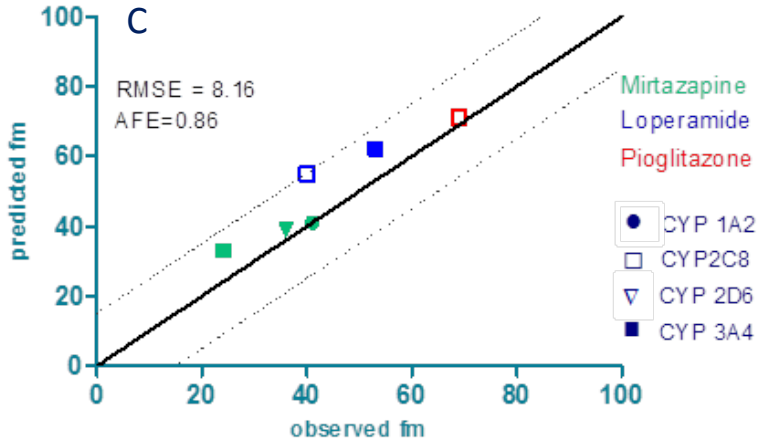
Recombinant CYPs RAF using nifedipine



Recombinant CYPs RAF using Testosterone



Silensomes™



When the 4 drugs were tested using Silensomes™, the calculated fm values for each CYP correlated very well with in vivo data (Fig C).

The contributions of different CYPs to the metabolism of mirtazapine, loperamide and lioglitazone were better predicted by Silensomes™ than using recombinant CYPs and the RAF approach.

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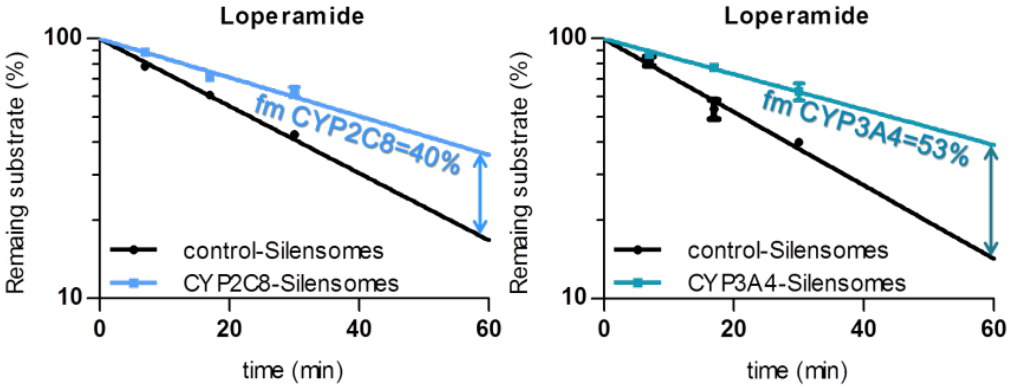
Silensomes™ – Multi-CYP substrates are easily identified and their respective fm determined

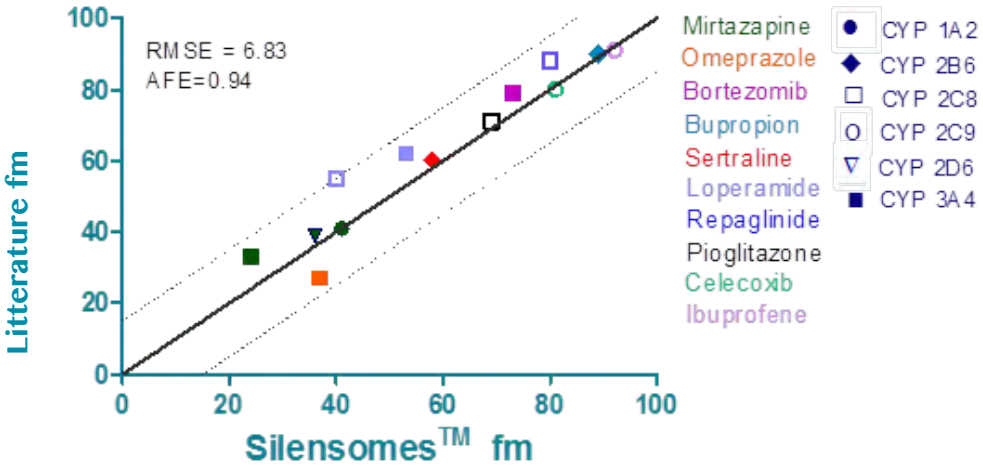


The intrinsic clearance of Loperamide was evaluated with Silensomes™ (CYP 1A2, 2B6, 2C8, 2C9, 2D6, 3A4) and their homologous controls.
Results confirmed that only CYP2C8 and CYP3A4 are involved in the metabolism - their contributions were accurately determined.

Correlation of the in vitro fm prediction using Silensomes™ and in vivo fm

10 multi-CYP substrates were incubated with qualified sets of CYP-Silensomes™ and homologous controls.

For all tested compounds, the in vitro CYP contributions (fm), obtained with Silensomes™, correlate well with the in vivo values.



Product references

Catalogue Number	SILENSOMES™
SIL210	Human hepatic CYP1A2-SILENSOMES™
SIL220	Human hepatic CYP2A6-SILENSOMES™
SIL230	Human hepatic CYP2B6 SILENSOMES™
SIL250	Human hepatic CYP2C8-SILENSOMES™
SIL260	Human hepatic CYP2C9-SILENSOMES™
SIL270	Human hepatic CYP2C19-SILENSOMES™
SIL240	Human hepatic CYP2D6-SILENSOMES™
SIL280	Human hepatic CYP2E1-SILENSOMES™
SIL200	Human hepatic CYP3A4-SILENSOMES™

Catalogue Number	Control-SILENSOMES™
SIL211	Human hepatic Control CYP1A2-SILENSOMES™
SIL221	Human hepatic Control CYP2A6-SILENSOMES™
SIL231	Human hepatic Control CYP2B6 SILENSOMES™
SIL251	Human hepatic Control CYP2C8-SILENSOMES™
SIL261	Human hepatic Control CYP2C9-SILENSOMES™
SIL271	Human hepatic Control CYP2C19-SILENSOMES™
SIL241	Human hepatic Control CYP2D6-SILENSOMES™
SIL281	Human hepatic Control CYP2E1-SILENSOMES™
SIL201	Human hepatic Control CYP3A4-SILENSOMES™

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