A HIGH THROUGHPUT SCREENING ASSAY FOR CYP3A4 INTERACTIONS USING SPA

[1]Alison Hopkins, [1]Kerry B. Howells, [1]Molly J. Price-Jones, [1]Kelvin T. Hughes, [2]Peter J. Eddershaw, [2]Steve R. Hood, [2]Amanda J. Woodrooffe, [2]Michael H. Tarbit.

[1]*Amersham Biosciences UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, England HP7 9NA [Telephone: 01222 526417, Fax 01222 526474,*

[2]*Bioanalysis and Drug Metabolism, GlaxoWellcome Research & Development, Ware, U.K.*

Introduction

Amersham Biosciences has developed an homogenous assay technology applicable to high throughput screening.
The Scintillation Proximity Assay (SPA) principle is based on the observation that in aqueous solution weakly-emitting β–isotopes such as [^{3}H] need to be close to scintillant molecules to produce a light signal. If not, the energy is dissipated in the aqueous solvent.

This SPA assay uses underivatised yttrium silicate (YSi) beads and is suitable for the measurement of CYP3A4 interactions through the inhibition of [^{3}H]ethynyloestradiol metabolism by recombinant CYP3A4, co-expressed with NADPH-cytochrome P450 reductase (CYPOR)[1]. Residual binding of the radioactive ligand is determined following incubation of the test compound with CYP3A4/CYPOR and NADPH (1mM) for up to 2 hours at 37°C.

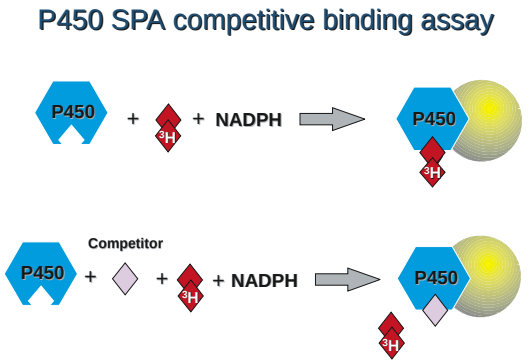

Figure 1 *P450 SPA assay concept*

Assay method

10µg CYP3A4 in 25µl PBS was pre-incubated with the test compound at 37°C for 15 minutes. This was followed by the addition of [^{3}H]ethynyloestradiol (0.1µCi/well, ~150-200,000 liquid scintillation counts) and 1mM NADPH (25µl each).
The reactions were further incubated for 2 hours at 37°C.
1mg YSi SPA beads was added to each well, the plate was shaken for 10 minutes and counted. Total assay volume was 150µl.
Results may be obtained for up to18 hours after the bead addition.

"Blanks" are microsomes prepared from mock-infected cells, this signal was subtracted from the P450 counts to give corrected specific SPA cpm. NSB (non-specific binding) was calculated from wells containing PBS, [^{3}H]ethynyloestradiol and NADPH only.

Results

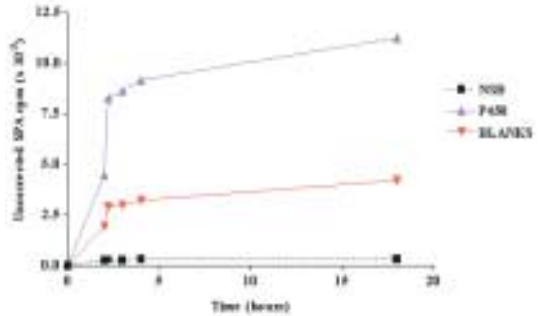

Figure 2 *Typical CYP3A4 assay results.*
The uncorrected P450 SPA signal generated was typically >5000 cpm. Signal:noise, calculated as the P450 SPA signal divided by NSB, was generally > 20:1.

ID	COMPOUND	P450	BLANK	CORR[a]	% OF CONTROL
	CONTROL	**10606**	**4701**	**5905**	**100**
1	SALMETEROL	7183	4097	**3086**	**52**
2	MIDAZOLAM	7540	4186	**3354**	**57**
3	TESTOSTERONE	13312	4184	**9128**	**155**
4	CHLORZOXAZONE	10123	4229	**5894**	**100**
5	BUDESONIDE	7356	4055	**3301**	**56**
6	QUINIDINE	6556	3984	**2572**	**44**
7	TOLBUTAMIDE	8998	4092	**4906**	**83**
8	GW-X	4952	4248	**704**	**12**
9	RANITIDINE	9098	4310	**4789**	**81**
10	PHENACETIN	9048	4303	**4745**	**80**
11	BUFURALOL	8859	4698	**4161**	**71**
12	ERYTHROMYCIN	4712	4978	**0**	**0**
	CONTROL	**9654**	**5169**	**4485**	**100**
13	aNF	12543	5006	**7537**	**168**
14	FURAFYLLINE	7322	5157	**2165**	**48**
15	DISULFIRAM	4876	5060	**0**	**0**
16	VERAPAMIL	5412	5142	**270**	**6**
17	THEOPHYLLINE	8486	5186	**3300**	**74**
18	HYDROXYPHENYL-PROPIONIC ACID	8610	5048	**3562**	**79**
19	ETOPOSIDE	10359	5093	**5266**	**117**
20	ACETOMINOPHEN	8119	5241	**2878**	**64**
21	FLUORESCEIN	6132	4770	**1362**	**30**
22	TAO	5038	5650	**0**	**0**
23	KETOCONAZOLE	4912	6357	**0**	**0**

Figure 3 *Screening results shown are from counting the plate immediately, and are replicates, (n=3).*

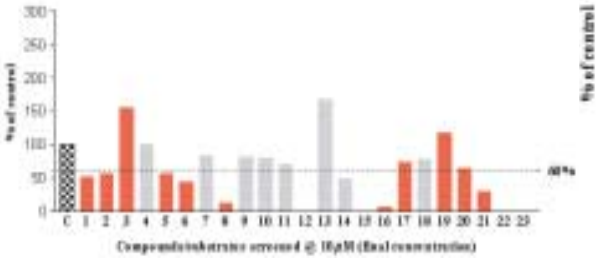

Figure 4 *The assay has been used to distinguish known CYP3A4 substrates from a series of P450 probe compounds.*

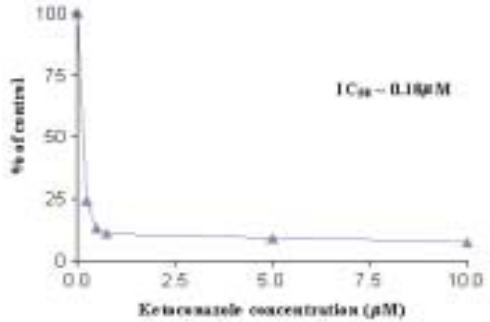

Figure 5 *Typical results achieved with ketoconazole, a potent selective inhibitor of CYP3A4.*

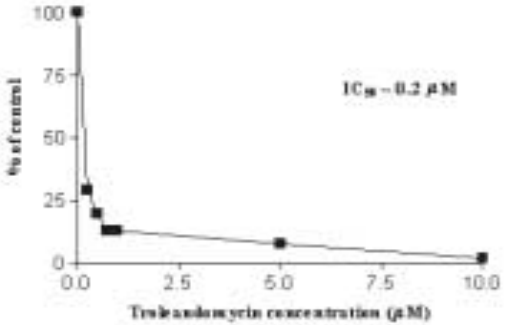

Figure 6 *Typical results achieved with troleandomycin, (TAO), a mechanism based inhibitor of CYP3A4.*

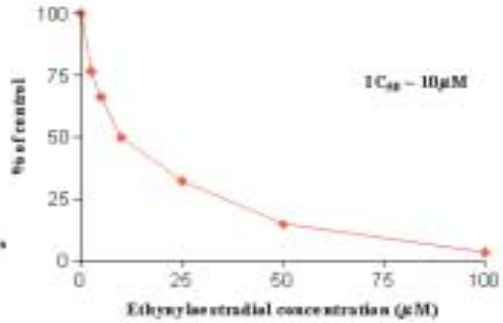

Figure 7 *Typical results achieved with ethynyloestradiol, a CYP3A4 substrate.*

This assay has been successfully used to distinguish known CYP3A4 substrates from a series of P450 probe compounds.

From a panel of 23 compounds, 11 out of 15 substrates were correctly identified as having interactions with CYP3A4[2].
Two substrates, testosterone and etoposide, appeared to activate [^{3}H]ethynyloestradiol metabolism in this system, a phenomenom previously reported for this isoform[3]. Seven of the remaining 8 non-substrates were correctly identified. αNF is a known activator of CYP3A4[2].

This assay can be used to generate IC_{50} values as shown for ketoconazole and troleandomycin, both well known inhibitors of CYP3A4[2].

Discussion

We have developed an SPA assay suitable for the measurement of CYP3A4 interactions which is suitable for rapid screening of compounds in microplate format for interactions with CYP3A4.

CYP3A4 is the major P450 isoform in the human liver and small intestine. It is responsible for the metabolism of both endogenous substrates such as steroid hormones (e.g. testosterone, oestradiol etc.) and a large number of exogenous drugs, dietary constituents and other xenobiotics.

Interactions with CYP3A4 can have important consequences for the development of new drugs through the metabolic fate of the compound itself and/or the effects on the disposition of co-administered therapeutics. The ability to optimise these properties is therefore an important part of the drug discovery process.

References

1. LEE,C.A., *et al.*, *Arch.Biochem.Biophys.* **319**,157-167, (1995).
2. PARKINSON, A., *Toxicologic Pathology*, **24(1)**, 45-57, (1996).
3. UENG, Y-F., *et al.*, *Biochemistry*, **36**, 370-381, (1997).

This poster has been produced as a result of a Technology Transfer collaboration between:

008908_30

PerkinElmer
For the Better