A HIGH THROUGHPUT SCREENING ASSAY FOR CYP3A4 INTERACTIONS USING SPA


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 [³H] 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 undetivatised yttrium silicate (YSi) beads and is suitable for the measurement of CYP3A4 interactions through the inhibition of [³H]etlynoestradiol metabolism by recombinant CYP3A4, co-expressed with NADPH-cytochrome P450 reductase (CYPOR)². 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.

Results
From a panel of 23 compounds, 11 out of 15 substrates were correctly identified as having interactions with CYP3A4. Two substrates, testosterone and etoposide, appeared to activate CYP3A4. Testosterone metabolism in this system, a phenomenon previously reported for this isoform.

This assay can be used to generate IC₅₀ values as shown for testosterone and troleandomycin, both well known inhibitors of CYP3A4.

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 isoenzyme in the human liver and small intestine. It is responsible for the metabolism of both endogenous substrates such as steroid hormones (e.g. testosterone, oestriol 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

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