Automated Cytochrome P450 Profiling

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Introduction

Cytochrome P450 enzymes play a central role in drug metabolism (1) and drug-drug interaction(2,3,4), and so identification of P450 liabilities at early stage of lead discovery should allow subsequent reduction in the attrition of drug candidates through the selection of templates void of this liability(5).

Here we describe an automated assay platform for expanded P450 profiling using established fluorescence-based P450 assays(6). The platform is composed of Polara™ robotic system integrated with dual Nanodrop™ dispensers and Acquro™ reader.

One operator can easily screen >1000 compounds in full dose-response curves for six P450 isoenzymes in 2 days. Excellent assay performance has been achieved with low failure rate. Aspects in assay validation, standard compound selection and quality control in assay performance and pharmacology are discussed.

Substrates and Enzymes

P450 Enzymes-
Recombinant human P450s baclosomes with co-expressed P450 reductase from Cypex Ltd., Dundee, UK. (7)

P450 Substrates-
• CYP3A4 VR assay: dealkylation of Vivid™ Red to yield resorufin;
• CYP3A4 VG assay: dealkylation of Vivid™ Green to yield rhodamine;
• 1A2: dealkylation of ethoxyresorufin to yield resorufin;
• 2C19: demethylation of SB-363399 to generate fluorescent coumarin;
• 2C9: demethylation of SB-355907 to generate coumarin;
• 2D6: demethylation of SB-285033 to generate coumarin.

Intra-run robustness measures

<table>
<thead>
<tr>
<th>Signal/background ratio</th>
<th>Z prime</th>
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<tbody>
<tr>
<td>3.4 A VR 1A2</td>
<td></td>
</tr>
<tr>
<td>3.4 A VG N1</td>
<td></td>
</tr>
<tr>
<td>3.4 A VG N2</td>
<td></td>
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<tr>
<td>3.4 A VG 1A2</td>
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Profiling of 36 source compound plates against each of six P450 enzymes. Signal/background ratio and Z primes values for sequential plates in 3 automation runs. Assays are grouped together according to plate reader setting and reagent stability, with assays having more reliable components running first.

There is no significant drop in assay performance (Z') during the runs.

Assay platform

• Two Nanodrop™ dispensers
• ThermoCRS Catalyst Express robotic workstation
• Plate storage carousel
• Robot arm
• Incubation hotel
• Acquro™ plate reader

• Throughput: 3 min per plate, 20/hour;
108 plates/run (reagent limited); Three enzymes can be combined in one run.

Z prime over time (from 11/2005 to 06/2006)

<table>
<thead>
<tr>
<th>Assay plate number</th>
<th>Z prime</th>
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<tbody>
<tr>
<td>1A2</td>
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<tr>
<td>2C19</td>
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<tr>
<td>2C9</td>
<td></td>
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<tr>
<td>2D6</td>
<td></td>
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<tr>
<td>3A4 VG</td>
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<tr>
<td>3A4 VR</td>
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<td>3A4 VG</td>
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Average Z'= 0.81±0.071, from 4270 assay plates. Average Z failure rate = 0.49%.

Correlation of duplicates (pC50)

A fully automated biochemical assay platform has been developed for in vitro profiling of six P450 isoenzymes. The platform produces excellent assay performance for all six isozymes, as measured by both robust Z' values and low week-to-week variability in the measured potency of standard compounds.

The performance of the automation and instrumentation has been extremely stable, providing full walk-away capability. The throughput and reliability has allowed a single operator to deliver profiling cycle times of less than one week.

During the course of this project, cumulative screening metrics have exceeded 5000 assay plates, 170,000 full dose-response curves, and 30,000 individual compounds. This screening activity now provides valuable data to numerous projects for use in selecting compounds with reduced late stage liabilities.

Acknowledgement

Supported by internal funding from GlaxoSmithKline.

We are very grateful for following individuals for their discussion, advice, and support in assays, automation, and data processing:


References