

CYP450 Inhibition Profiling Using High Throughput RapidFire Mass Spectrometry Assays

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ABSTRACT

Early assessment of ADME (Absorption, Distribution, Metabolism, and Excretion) properties of drug candidates has become an essential component of drug discovery. ADME characterizations are important in identifying compounds that are likely to fail in clinical development, meanwhile prioritizing candidates that are more likely to have good human pharmacokinetic properties and to avoid or minimize potential drug-drug interactions. At Exelixis we have established the capability to profile compounds (>100 compounds/week) in a panel of ADME assays in parallel with biochemical and cellular characterization.

Cytochrome P450 enzymes catalyze the biotransformation of the majority of xenobiotics in the liver and other tissues. The predominant drug metabolizing CYP450 isozymes are CYP3A4, CYP2D6, CYP1A2, CYP2C9, CYP2C19, and CYP2C8. We have established LC-MS methods to assay these CYP450 isozymes expressed in human liver microsomes using specific drug substrates. Recently we integrated a RapidFire system with an ABI3000 mass spectrometer to increase throughput of these assays. This system has enabled a real-time and quantitative measurement of CYP450 inhibition in a dose-response manner, providing a rapid evaluation of potential clinically important drug-drug interactions. The assay development, process, and CYP450 results obtained with the RapidFire system will be presented.

FIGURE 1: RapidFire System Layout

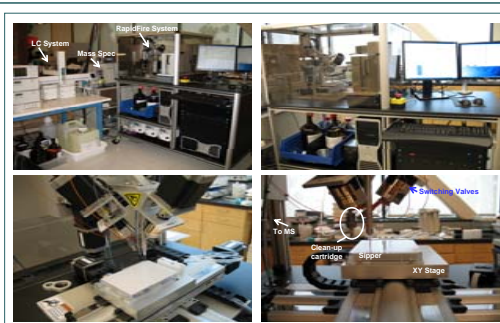


FIGURE 2: Example MS Signals

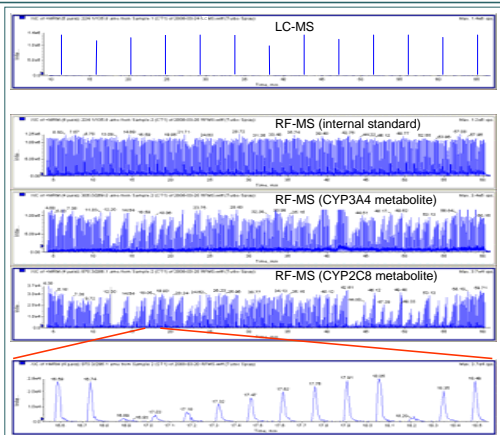


Figure 2. RapidFire RF-MS system, developed by BioTrove, replaces HPLC with a rapid sample purification system using micro scale solid-phase extraction (mSPE) technology. It has a throughput of 5-10 seconds per sample, comparing to 4-5 minutes per sample from conventional LC-MS systems.

FIGURE 3: Correlation Study for RapidFire Evaluation

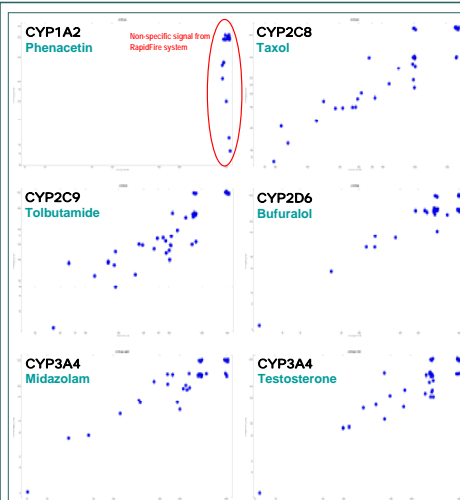


Figure 3. Fifty compounds, including known CYP450 inhibitors, were selected. Assay samples were evaluated on both conventional LC-MS and RapidFire RF-MS systems. Good correlations were observed from both systems, except CYP1A2. Phenacetin, substrate for CYP1A2, requires a LC separation step due to non-specific MS signal. A different substrate for CYP1A2 needs to be used for RapidFire system.

FIGURE 4: Substrate Study for CYP1A2

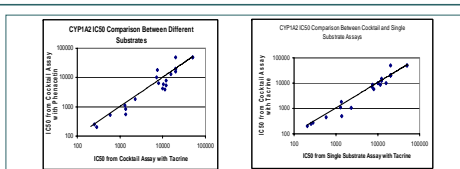


Figure 4. Tacrine is an acceptable substrate for CYP1A2, according to FDA guidance. Twenty-four compounds were selected randomly, and evaluated in CYP1A2 inhibition assay with either Phenacetin (LC-MS) or Tacrine (RF-MS) as substrate. Good correlations were observed between IC_{50} values generated in the two assays. In addition, Tacrine returned comparable IC_{50} values in both cocktail and single substrate formats (RF-MS).

FIGURE 5: RapidFire CYP450 Inhibition Assay Scheme

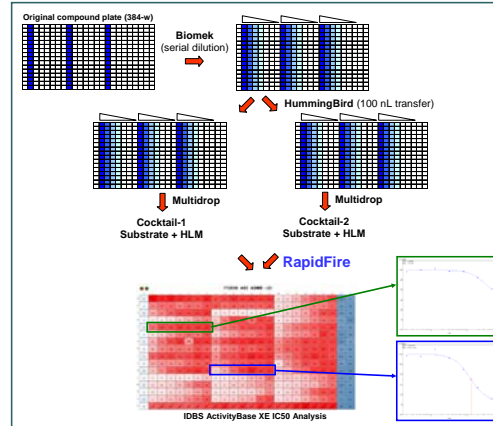


FIGURE 6: Flow-chart of CYP450 Inhibition Studies

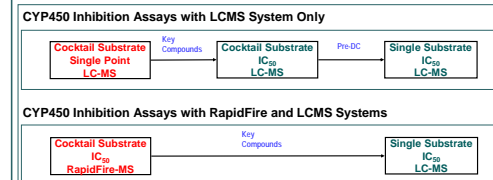
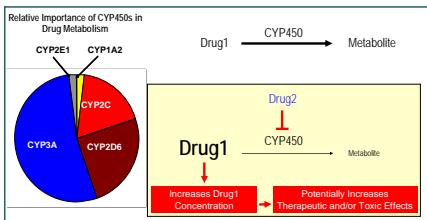


Figure 6. The higher throughput from RapidFire system enables us to bypass single point evaluation, and to generate CYP450 IC_{50} values for all Exelixis medchem compounds in the discovery projects.

CONCLUSION

We have successfully integrated a RapidFire system with an ABI-3000 mass spectrometer for CYP450 inhibition assays. RF-MS provides data quality comparable to LC-MS systems, but with a ~30-fold increase in speed. This increased throughput enables quantitative analyses of CYP450 inhibition in a 7-point dose-response format. Subsequent SAR can be established early in the lead optimization stage. RapidFire system also can help identify possible drug-drug interactions at an early stage of drug development.

Drug-Drug Interaction by CYP450 Inhibition



Conventional LC-MS vs. RapidFire RF-MS

Conventional LC-MS

- Mass-based quantitation of selected analytes in complex mixtures (sensitive and selective)
- Increased selectivity from a separation step before mass spec analysis
- Relatively low throughput (minutes/sample) with a long separation step

RapidFire RF-MS from BioTrove

- High throughput (seconds/sample) with a rapid sample purification step
- Micro scale solid-phase extraction (mSPE)
- Compatible with 96-well and 384-well sample plates
- Compatible with many biological matrices
- Some CYP450 substrates may not be suitable