High-Throughput *In Vitro* ADME Analysis with Agilent RapidFire/MS Systems: Cytochrome P450 Inhibition

Application Note

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Introduction

Analysis of cytochrome P450 (CYP) inhibition is an important component of the drug discovery process as adverse drug-drug interactions can lead to termination of a drug development program, withdrawal from the market, or restrictions on therapeutic use. The desire to eliminate weak candidates at earlier phases of the drug discovery process has caused *in vitro* ADME analysis to shift earlier as well, resulting in the need to evaluate a larger number of samples. Therefore, an efficient means of analyzing cytochrome P450 assays in a fast and cost-effective manner is required. Agilent RapidFire/MS systems combine high-throughput sample processing with triple quadrupole (QQQ) or time-of flight (TOF) mass spectrometry (MS) to streamline ADME assay analysis.

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Direct Cytochrome P450 Inhibition

RapidFire/MS systems have been validated to use FDA recommended drug probes for cytochrome P450 assays (Table 1). Direct CYP inhibition experiments were performed in the following manner: IC_{50} values for a range of positive control inhibitors (seven non-zero inhibitor concentrations) were determined in individual incubations of pooled human liver microsomes (HLM) using FDA preferred or acceptable drug probe substrates with previously validated assay methods[1]. Stably labeled isotope internal standards were used for probe substrate metabolites. Samples were analyzed individually on a RapidFire/MS/MS system and by traditional LC/MS/MS. Correlation of results are shown in Figure 1, and indicate that the RapidFire system produces equivalent results to LC/MS/MS[2]. The RapidFire run time was approximately 6 s per sample, compared to 2-4 min for LC/MS/MS, providing an efficient and productive means to evaluate cytochrome P450 assays within existing laboratory workflows.

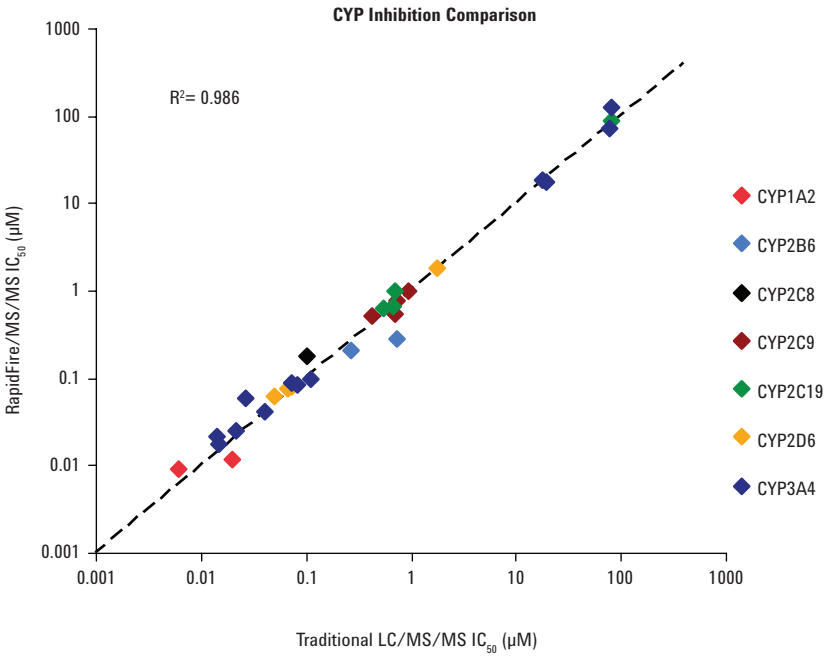

Figure 1. Correlation of IC_{50} values obtained by traditional LC/MS/MS and RapidFire/MS/MS. Data represent eight different enzyme/substrate pairs with one to three inhibitors for each. The dotted line represents a line of unity.

Table 1. RapidFire/MS systems can analyze FDA-recommended drug probes.

CYP1A2	tacrine, melatonin
CYP2B6	bupropion
CYP2C8	amodiaquol, taxol
CYP2C9	tolbutamide, diclofenac
CYP2C19	S-mephentoin, omeprazole
CYP2D6	dextromethorphan, bufuralol
CYP2E1	chlorzoxazone
CYP3A4/5	midazolam, testosterone, nifedipine, erthromycin

Time-Dependent Cytochrome P450 Inhibition

Time-dependent CYP inhibition experiments were performed using similar methods[1]. IC_{50} values were calculated from seven-point curves for a range of inhibitors using the standard dilution approach, in duplicate individual incubations in HLMs, using FDA preferred CYP3A4 drug probe substrates (midazolam and testosterone). Correlation of IC_{50} results are shown in Figure 2, and again indicate that the RapidFire/MS/MS system produces equivalent results to LC/MS/MS[3] while increasing the productivity of assay analysis approximately 20-fold.

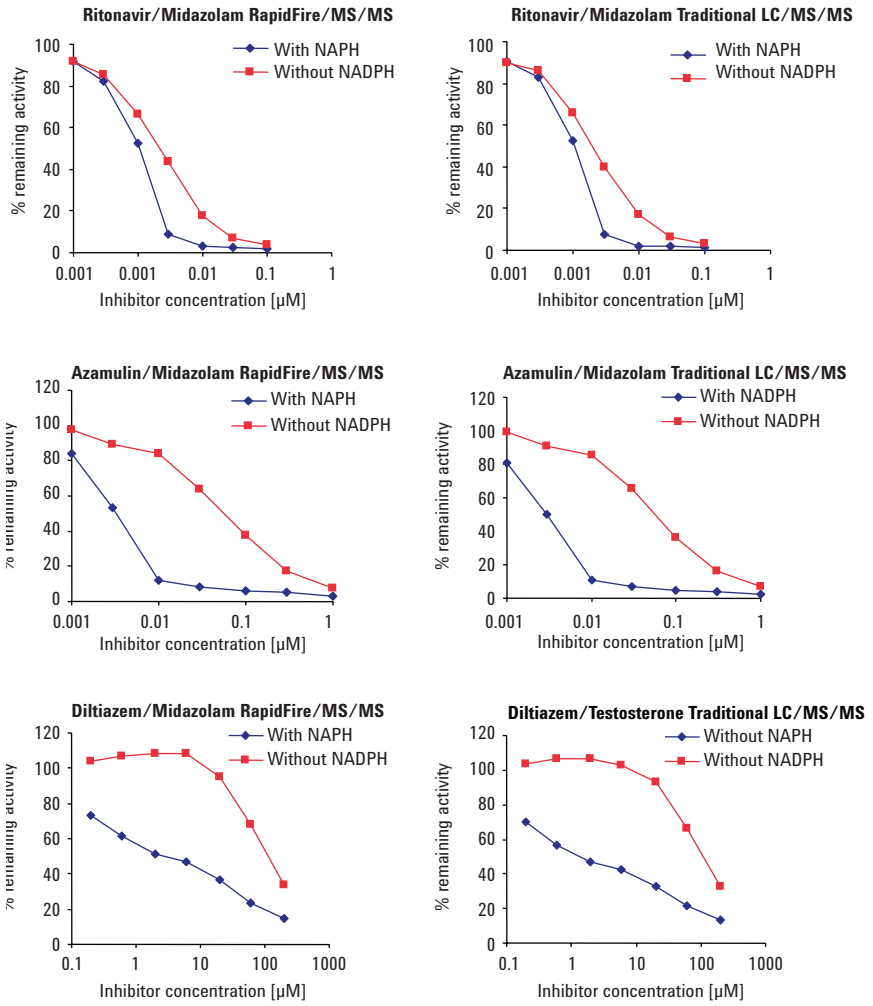

Figure 2. A comparison of IC_{50} values (time-dependent inhibition) for a range of inhibitors, using CYP3A4 drug probe substrates with RapidFire/MS/MS and traditional LC/MS/MS methods, shows equivalent results. However, a full IC_{50} curve (seven points) can be analyzed with RapidFire/MS/MS in less time than a single LC/MS/MS data point.

Conclusions

The Agilent RapidFire High-throughput Mass Spectrometry system was used to analyze the cytochrome P450 inhibition assay. RapidFire methods for CYP inhibition have been developed for the standard FDA approved substrate molecules. The results from two sets of experiments illustrate the key benefits of the RapidFire System compared to conventional LC/MS methods: a greater than 10-fold increase in throughput (6-10 s processing time per sample), and equivalent inhibition results (IC_{50} values). As a result, the RapidFire system significantly increased the efficiency and throughput of conventional laboratory workflows for these assays.

References

1. Perloff, E. *et al*. Validation of cytochrome P450 time-dependent inhibition assays: a two-time point IC_{50} shift approach facilitates kinact assay design. *Xenobiotica,* **2011**, 39(2):99-112.
2. Perloff, E. *et al*. Comparison of RapidFire Ultra High Throughput LC/MS/MS with Traditional LC/MS/MS for Cytochrome P450 Inhibition Testing. *Abstract #106*, Presented at the 11th European ISSX Meeting, May 17-20, **2009**, Lisbon, Portugal.
3. Miller, V. *et al*. Evaluation of High Throughput Screening Methods for Time-Dependent Inhibition of CYP3A4 Utilizing RapidFire LC/MS/MS Technology. Presented at the 11th European ISSX Meeting May 17-20, **2009**, Lisbon, Portugal.

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