Application Note

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High-Throughput *In Vitro* ADME Analysis with Agilent RapidFire/MS Systems

A streamlined cytochrome P450 inhibition assay

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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.

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 seconds per sample, compared to 2 to 4 minutes for LC/MS/MS, providing an efficient and productive means to evaluate cytochrome P450 assays within existing laboratory workflows.

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

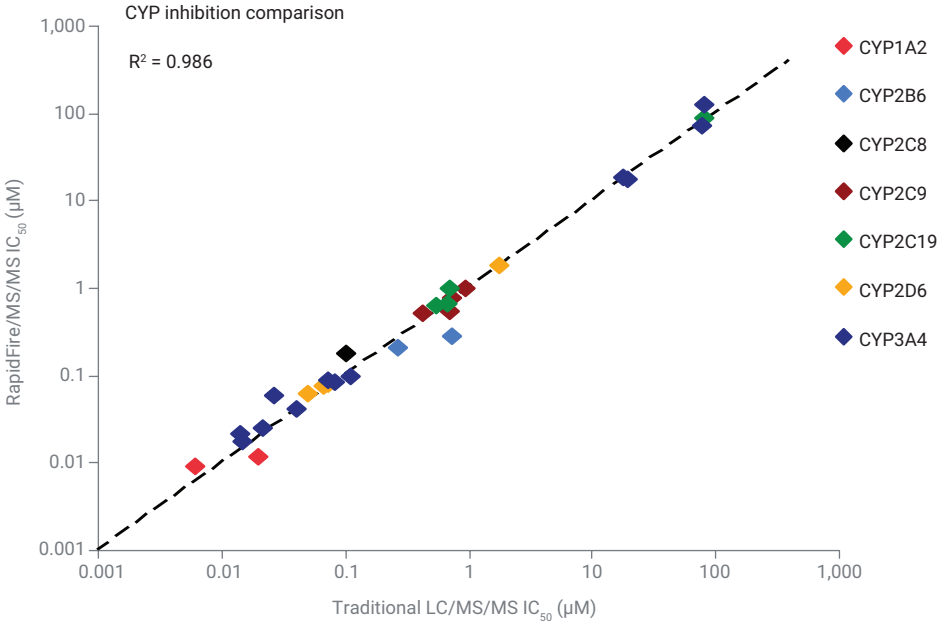

Figure 1. Correlation of IC_{50} values obtained by traditional LC/MS/MS and RapidFire/MS/MS. Data represent eight different enzyme/substrate pairs with one to three inhibitors for each. The dotted line represents a line of unity.

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.

Conclusion

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 to 10 seconds 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.

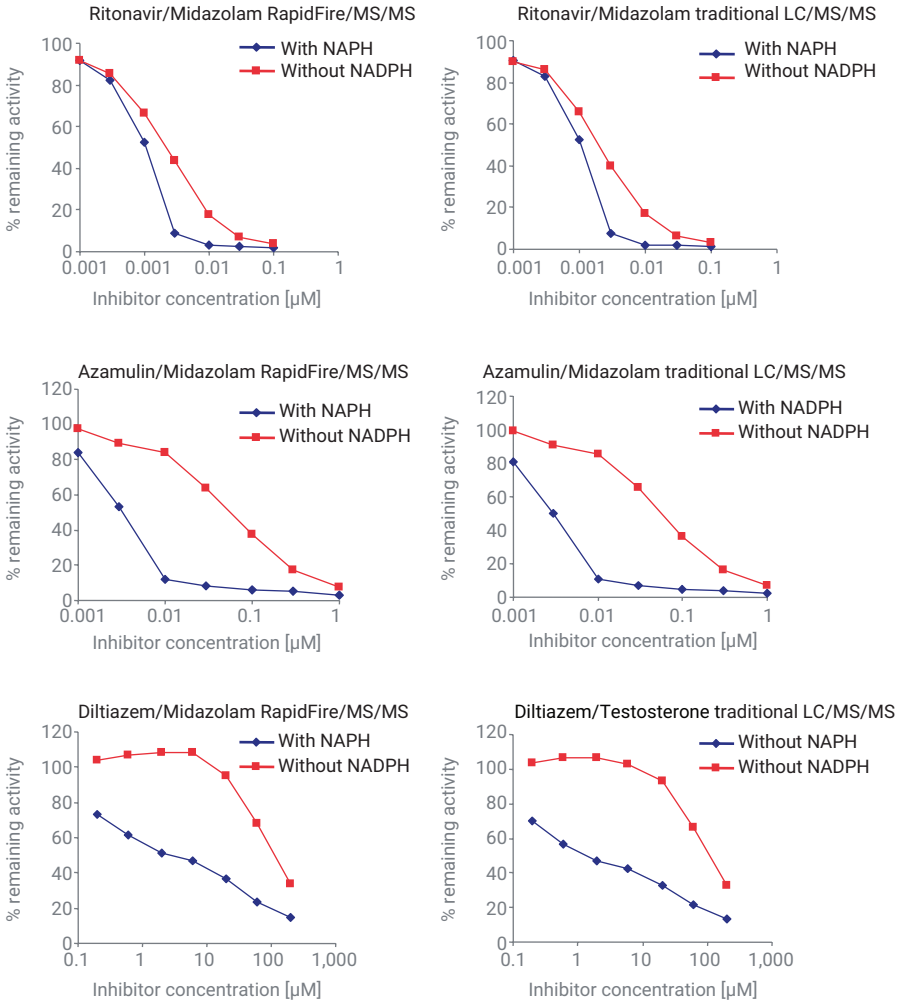

Figure 2. A comparison of IC_{50} values (time-dependent inhibition) for a range of inhibitors, using CYP3A4 drug probe substrates with RapidFire/MS/MS and traditional LC/MS/MS methods, shows equivalent results. However, a full IC_{50} curve (seven points) can be analyzed with RapidFire/MS/MS in less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

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 Inhibitionof CYP3A4 Utilizing RapidFire LC/MS/MS Technology. Presented at the 11th European ISSX Meeting May 17–20, **2009**, Lisbon, Portugal.

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