

# Consistent High-throughput CYP Inhibition Data Across Mass Spectrometry Platforms

William A. LaMarr(1), Michelle V. Romm(1), Nikunj Parikh(1), Vaughn Miller (1), Can C. Ozbal (1)  
 (1)BioTrove, Inc., Woburn, MA, <http://www.biotrove.com>



## Abstract

Cytochrome P450s (CYP) mediate the biotransformation of drug compounds. Metabolism of multiple test compounds by the same CYP isoform can alter reaction kinetics and result in adverse drug-drug interactions (DDI). Therefore, DDI assays are valuable tools in the go/no-go decision-making process in drug discovery. Mass spectrometry (MS)-based CYP inhibition assays have emerged as the preferred method in early drug discovery because it allows for the use of human liver microsomes and drug probe substrates. However, conventional LC-MS requires several minutes per sample processing time creating a bottleneck in drug discovery screening. Using the RapidFire system, we have developed analytical techniques which lower analysis times to only a few seconds per sample, allowing DDI assays to be performed at throughputs consistent with other drug discovery screening platforms. In the present study we evaluated the compatibility of the RapidFire system with the major MS platforms (ABI/Sciex, ThermoFisher, & Agilent) by measuring CYP enzyme kinetics for 4 isoform/probe pairs using conventional assay methods. The Km values measured across all isoforms and MS platforms tested were within a 2-fold window and were consistent with previously published literature. The IC50 values for several traditional CYP inhibitors were statistically equivalent and in line with literature values. This data shows that the RapidFire system produces consistent, high-throughput, CYP inhibition data across multiple MS platforms enabling performance of these critical assays in a typical drug discovery environment.

## Assay Conditions

\* All assays were monitored using the RapidFire high-throughput mass spectrometry system software interfaced to an Agilent 6410, an Applied Biosystems Sciex AP4000, and a Thermo Scientific TSQ Quantum Ultra triple quadrupole mass spectrometer.

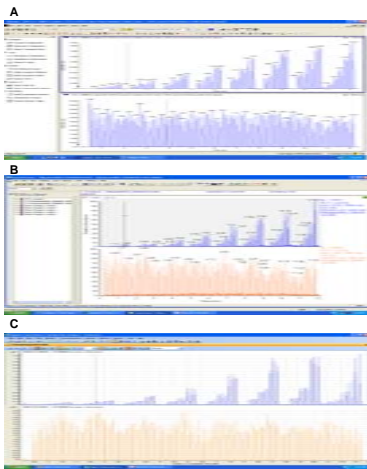
### RapidFire Conditions

- \* Buffer A = Water with 0.09% formic acid, 0.01% TFA; 1.5 mL/min flow rate
- \* Buffer B = 100% acetonitrile with 0.09% formic acid, 0.01% TFA; 1.0 mL/min flow rate
- \* SPE Column A (reversed-phase C<sub>8</sub> chemistry)

\* The product and its internal standard were monitored simultaneously in all experiments

Substrate	Product	Q1	Q3
3A4 - Midazolam	1'-hydroxymidazolam	342.1	203.1
3A4 - Testosterone	6β-hydroxytestosterone	305.3	289.5
2C9 - Diclofenac	4'-hydroxydiclofenac	312.1	231.0
2D6 - Dextromethorphan	dextrorphan	258.1	157.0

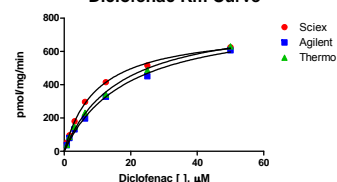
## RapidFire System



Screen shots of Diclofenac Km Curve on (A) Sciex (B) Thermo (C) Agilent

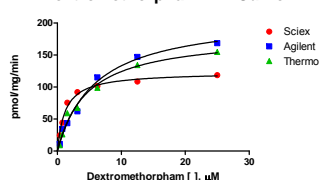
## Results

### Diclofenac Km Curve



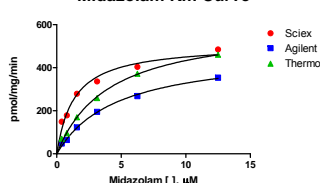
The activity of CYP2C9 was validated using Diclofenac as a probe. Using non-linear curve fitting software (GraphPad Prism), the Km and Vmax values were calculated and the Km values are within the ranges (3.4-52 µM) recommended by the FDA.

### Dextromethorphan Km Curve



The activity of CYP2D6 was validated using Dextromethorphan as a probe. Using non-linear curve fitting software (GraphPad Prism), the Km and Vmax values were calculated and the Km values are within the ranges (0.44-8.5 µM) recommended by the FDA.

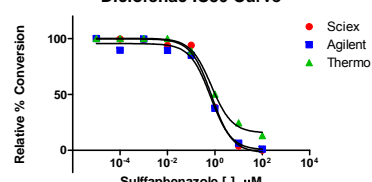
### Midazolam Km Curve



The activity of CYP3A4 was validated using Midazolam as a probe. Using non-linear curve fitting software (GraphPad Prism), the Km and Vmax values were calculated and the Km values are within the ranges (1-14 µM) recommended by the FDA.

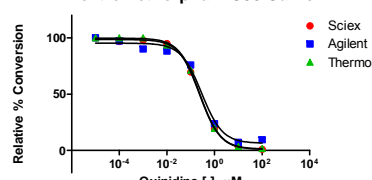
## Results

### Diclofenac IC50 Curve



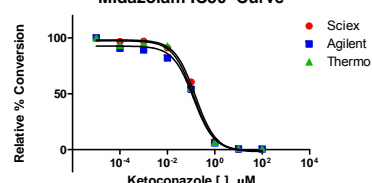
An IC50 curve for Sulfaphenazole, a known inhibitor of CYP2C9, was run using optimized conditions: 0.25mg/ml HLM, 15µM Diclofenac, 45 min incubation at 30°C, quenched with an equal volume of acetonitrile containing internal standard. The calculated IC50 values are close to values reported in literature.

### Dextromethorphan IC50 Curve



An IC50 curve for Quinidine, a known inhibitor of CYP2D6, was run using optimized conditions: 0.25mg/ml HLM, 5µM Dextromethorphan, 45 min incubation at 30°C, quenched with an equal volume of acetonitrile containing internal standard. The calculated IC50 values are close to values reported in literature.

### Midazolam IC50 Curve



An IC50 curve for Ketoconazole, a known inhibitor of CYP3A4, was run using optimized conditions: 0.25mg/ml HLM, 6µM Midazolam, 10 min incubation at room temp (24°C), quenched with an equal volume of acetonitrile containing internal standard. The calculated IC50 values are close to values reported in literature.

## Results

### Comparison of Km values

	Agilent	AB Sciex	Thermo	Literature*
2C9 - Diclofenac	15.01 ± 1.68	9.165 ± 0.2599	13.70 ± 1.482	3.4 - 52
2D6 - Dextromethorphan	5.58 ± 0.45	3.61 ± 0.19	5.27 ± 0.17	0.44 - 8.5
3A4 - Midazolam	4.68 ± 0.62	1.99 ± 0.32	3.99 ± 0.43	1 - 14
3A4 - Testosterone	41.14 ± 17.28	71.18 ± 16.84	70.25 ± 23.51	52 - 94

### Comparison of Vmax values

	Agilent	AB Sciex	Thermo	Literature**
2C9 - Diclofenac	822.8	736.9	825.6	1670
2D6 - Dextromethorphan	211.9	123.1	182.1	202
3A4 - Midazolam	480.4	509.3	607.3	1220

### Comparison of IC50 values

	Agilent	AB Sciex	Thermo	Literature**
2C9 - Diclofenac (Sulfaphenazole)	0.66	0.73	0.58	0.27 - 0.75
2D6 - Dextromethorphan (Quinidine)	0.29	0.23	0.23	0.02 - 0.68
3A4 - Midazolam (Ketoconazole)	0.13	0.15	0.12	0.09 - 0.24
3A4 - Testosterone (Ketoconazole)	0.32	0.24	0.28	0.03 - 0.3

\* FDA Guidance for Industry, Drug Interaction Studies, 9/2006

\*\* Walsky and Obach, *Drug Metab Dispos.* 2004, 32(6):647-650. Di et al. *Internat J. Pharmaceutics.* 2007, 335:1-110.  
 Kim et al. *Rapid Commun. Mass Spectrom.* 2005, 19:2651-2658. Franklin et al. *Drug Metab. Rev.* 2007, 39:309-322.

## Conclusions

- Compatible with all major P450 isoforms
- Integrates with MS from multiple vendors
- Throughput 6-8 seconds/sample
- Label-free, native technology provides biologically relevant data