

Comparison of RapidFire® Ultra High Throughput LC/MS/MS with Traditional LC/MS/MS for Cytochrome P450 Inhibition Testing



Elke S. Perloff¹, Shangara S. Dehal¹, Andrew K. Mason¹, Andrew P. Blanchard¹, William A. LaMarr², Can C. Ozbal², Vaughn P. Miller², Charles L. Crespi¹, and David M. Stresser¹

¹BD Biosciences Discovery Labware, BD GentestSM Contract Research Services, Woburn, MA 01801, USA

²BioTrove, Inc. 10P Gill St. Woburn, MA 01801, USA



Introduction

Assessment of cytochrome P450 inhibition has moved into earlier phases of drug discovery, and recent guidance from the FDA recommends routine *in vitro* IC₅₀ determinations for at least six P450 isoforms. The resulting increase in the number of samples generated in P450 inhibition screens has led to a demand for higher throughput analysis options.

The objective of this study was to apply RapidFire® LC/MS/MS technology (BioTrove, Woburn, MA) to *in vitro* cytochrome P450 inhibition testing and compare the results to traditional LC/MS/MS methods validated in house (BD GentestSM Contract Research Services, Woburn, MA). In addition, the benefit of including a standard curve with an IC₅₀ sample set was investigated by comparing IC₅₀ values obtained based on peak area ratios with IC₅₀ values obtained based on analyte concentrations derived from 7-point standard curves.

Methods

IC₅₀ values (7 non-zero inhibitor concentrations) for a range of positive control inhibitors were determined in individual incubations in pooled human liver microsomes (BD Gentest, Woburn, MA) using FDA preferred or acceptable drug probe substrates with previously validated assay methods [1, 2], which are summarized in Table 1.

T1 Incubation Methods

Enzyme	Substrate	Metabolite	Incubation time	HLM mg/mL
CYP1A2	Phenacetin (40 uM)*	Acetaminophen	10 min	0.2
CYP1A2	Tacrine (5 uM)**	Hydroxytacrine	10 min	0.3
CYP2B6	Bupropion (80 uM)	Hydroxybupropion	5 min	0.1
CYP2C8	Amodiaquine (2 uM)	Desethylamodiaquine	5 min	0.02
CYP2C9	Diclofenac (5 uM)	4'-Hydroxydiclofenac	5 min	0.05
CYP2C19	(S)-Mefenphenytoin (40 uM)	4'-Hydroxymefenphenytoin	10 min	0.3
CYP2D6	Dextromethorphan (5 uM)	Dextrorphan	5 min	0.1
CYP3A4	Midazolam (3 uM)	1'-Hydroxymidazolam	5 min	0.02
CYP3A4	Testosterone (50 uM)	6β-Hydroxytestosterone	10 min	0.05

*LCMS, **RapidFire

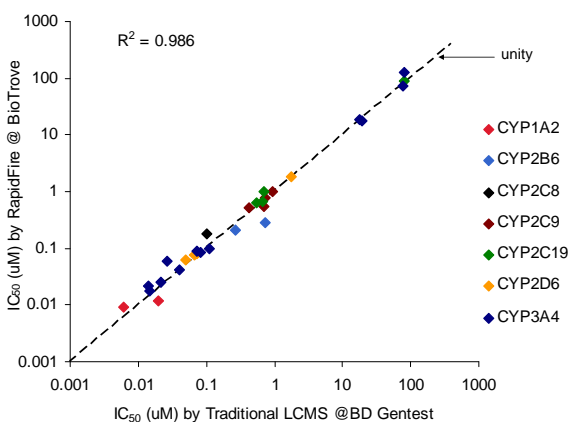
Stable-labeled isotope internal standards (BD Gentest, Woburn, MA) were used for all probe substrate metabolites except for 1-hydroxytacrine, where buceitin was employed. Samples were analyzed individually by RapidFire technology as well as by traditional LC/MS/MS methods.

Traditional LC/MS/MS methods were developed and validated at BD Gentest using an Applied Biosystems API4000 instrument and a 2-4 min solvent gradient on a Waters™ Symmetry™ C18 column [2].

RapidFire ultra high throughput LCMS methods were developed and run on a BioTrove RapidFire microscale solid-phase extraction preparation system interfaced to an ABI-4000 triple-quadrupole mass spectrometer with an average sample cycle time of 7 seconds.

Percent remaining activity was calculated for each inhibitor concentration, and IC₅₀ values were determined by linear interpolation. Standard curves, where applicable, were fit to linear or quadratic models with or without weighting using SigmaPlot v.8 (SPSS, Inc.). The most simple model resulting in 70%-130% accuracy (65%-135% for the LLOQ) was selected and used to calculate metabolite concentrations.

F1 Correlation between RapidFire and traditional LC/MS/MS



T2 RapidFire vs. Traditional LC/MS/MS IC₅₀ Values

Enzyme	Substrate	Inhibitor	IC ₅₀ (uM) ^a		Ratio BioTrove/BD
			Trad. LCMS BD	RapidFire BioTrove	
CYP1A2	Phenacetin/Tacrine	7,8-Benzoflavone	0.020	0.012	0.60
CYP1A2	Phenacetin/Tacrine	7,8-Benzoflavone	0.006	0.009	1.50
CYP2B6	Bupropion	Ketoconazole	0.72	0.28	0.39
CYP2B6	Bupropion	Ticlopidine	0.27	0.21	0.78
CYP2C19	S-Mefenphenytoin	S-Benzylirivanol	0.67	0.68	1.01
CYP2C19	S-Mefenphenytoin	S-Benzylirivanol	0.54	0.62	1.15
CYP2C19	S-Mefenphenytoin	S-Benzylirivanol	0.70	0.98	1.40
CYP2C19	S-Mefenphenytoin	S-Fluoxetine	81	87	1.07
CYP2C8	Amodiaquine	Montelukast	0.10	0.18	1.80
CYP2C9	Diclofenac	Sulfaphenazole	0.69	0.54	0.78
CYP2C9	Diclofenac	Sulfaphenazole	0.43	0.51	1.19
CYP2C9	Diclofenac	Sulfaphenazole	0.72	0.77	1.07
CYP2C9	Diclofenac	Tienillic Acid	0.94	1.00	1.06
CYP2D6	Dextromethorphan	Quinidine	0.068	0.076	1.12
CYP2D6	Dextromethorphan	Quinidine	0.049	0.064	1.31
CYP2D6	Dextromethorphan	Quinidine	0.073	0.082	1.13
CYP2D6	Dextromethorphan	Paroxetine	1.8	1.8	1.04
CYP3A4	Midazolam	Ketoconazole	0.071	0.087	1.23
CYP3A4	Midazolam	Ketoconazole	0.015	0.018	1.20
CYP3A4	Midazolam	Ketoconazole	0.027	0.059	2.21
CYP3A4	Midazolam	Azaminin	0.11	0.10	0.91
CYP3A4	Midazolam	Verapamil	18	19	1.05
CYP3A4	Midazolam	Diltiazem	80	124	1.55
CYP3A4	Midazolam	Diltiazem	0.021	0.026	1.20
CYP3A4	Testosterone	Ketoconazole	0.041	0.041	1.00
CYP3A4	Testosterone	Ketoconazole	0.014	0.022	1.57
CYP3A4	Testosterone	Azaminin	0.081	0.084	1.04
CYP3A4	Testosterone	Verapamil	19	18	0.92
CYP3A4	Testosterone	Diltiazem	78	71	0.92

^aAssays were performed according to [2]. IC₅₀ values are means of intra-plate duplicates calculated based on peak area ratio. Multiple data sets for the same enzyme/inhibitor pair were generated on different days.

Results

IC₅₀ values obtained using RapidFire ultra high throughput LC/MS/MS analysis were consistent with the data obtained using traditional LC/MS/MS methods validated in house (R² = 0.986, Figure 1). Greater than 55% of corresponding IC₅₀ values were within 1.2-fold, and >90% were within 2-fold of each other (Table 2).

A run time of approximately 7 seconds per injection with RapidFire technology compared to 2 to 4 minutes for traditional LC/MS/MS methods provided a significant (>20-fold) decrease in analysis time.

Standard curves generated by RapidFire analysis were best described using weighted (1/y or 1/y²) linear regression (SigmaPlot v.8). IC₅₀ values generated based on peak area ratios alone ranged from 99.8% to 126% of IC₅₀ values obtained using metabolite standard curves (Table 3).

T3 IC₅₀ values calculated with and without standard curves

Enzyme	Substrate	Inhibitor	IC ₅₀ value [uM]		Deviation from std curve IC ₅₀
			Based on Area ratio	Based on Standard curve	
CYP1A2	Tacrine	7,8-Benzoflavone	0.0092	0.0092 linear 1/y	0.7%
CYP2B6	Bupropion	Ketoconazole	2.95	2.34 linear 1/y	26.2%
CYP2C8	Amodiaquine	Montelukast	0.058	0.057 linear 1/y ²	2.5%
CYP2C9	Diclofenac	Sulfaphenazole	0.34	0.34 linear 1/y	-0.2%
CYP2C19	(S)-Mefenphenytoin	S-Benzylirivanol	0.85	0.81 linear 1/y ²	4.7%
CYP2D6	Dextromethorphan	Quinidine	0.047	0.047 linear 1/y ²	0.6%
CYP3A4	Midazolam	Ketoconazole	0.027	0.027 linear 1/y	0.4%
CYP3A4	Testosterone	Ketoconazole	0.022	0.021 linear 1/y	3.4%

IC₅₀ values are means of intra-plate duplicates.

Conclusions

- Cytochrome P450 inhibition IC₅₀ results obtained using RapidFire analysis were comparable to those obtained using validated traditional LC/MS/MS methods (>90% of values within 2-fold of each other).
- The increased analysis speed represents a >20-fold improvement in cycle time, thereby permitting rapid data delivery to project teams and clients. Alternatively, ultra-rapid analysis allows acquisition of more data points per unit time providing for the option of conducting robust, multipoint assays typical of drug development.
- IC₅₀ values generated using RapidFire analysis with and without standard curves (weighted linear regression) were comparable (within 30% of each other), suggesting that standard curves may be omitted in RapidFire discovery IC₅₀ screens to further improve turnaround time without compromising data quality.

References

- Draft Guidance for Industry: Drug Interaction Studies — Study Design, Data Analysis, and Implications for Dosing and Labeling (www.fda.gov/cder/guidance/6695dft.pdf)
- Perloff ES, et al. Xenobiotica 39:99 (2009)