

Evaluation of accurate mass TOF-MS for use in high throughput CYP450 inhibition screening



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Introduction

Cytochrome P450s (CYP450) mediate the biotransformation of drug compounds. Metabolism of multiple test compounds by the same CYP450 isoform can alter reaction kinetics and result in adverse drug-drug interactions (DDI). Therefore, high throughput-DDI assays are valuable tools in the go/no-go decision-making process in drug discovery. Mass spectrometry-based CYP450 assays have emerged as the preferred method in early discovery due to their sensitivity, specificity, and robustness. Traditionally DDI assays utilize tandem MS, a process which is rate limited by its requisite method development. The use of accurate mass detection, offered by time of flight (TOF) mass spectrometers, may provide an alternative to tandem-MS. The current work compares triple quadrupole (QqQ)-MS to TOF-MS for the measurement of traditional p450 inhibition samples.

Exact mass MS for high-throughput CYP450 inhibition and other *in vitro* ADME applications

Assay Conditions

All assays were monitored using the RapidFire high-throughput mass spectrometry system interfaced to an Agilent 6220 Accurate-Mass TOF and an Applied Biosystems Sciex API4000 triple quadrupole mass spectrometer.

RapidFire Conditions

Buffer A = 100% water with 0.09% formic acid, 0.01% TFA

Buffer B = 100% acetonitrile with 0.09% formic acid, 0.01% TFA

SPE Column A (reversed-phase C₄ chemistry)

The product and its internal standard were monitored simultaneously in all experiments. Below are the transitions used on the API4000.

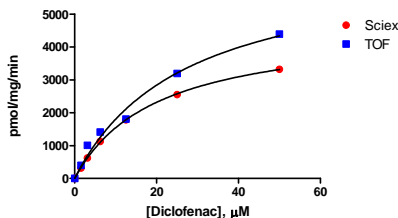
Substrate	Analyte	Q1	Q3
3A4 - Midazolam	1-Hydroxymidazolam	342.1	203.1
	(¹³ C) ₄ -1-Hydroxymidazolam	346.1	207.1
2C9 - Diclofenac	4-Hydroxydiclofenac	312.1	231.0
	(¹³ C) ₄ -4-Hydroxydiclofenac	318.1	237.0
2D6 - Dextromethorphan	Dextrophan	258.1	157.0
	Dextrophan-d ₃	261.3	157.0

RapidFire System



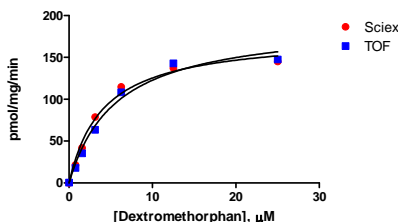
Results

Diclofenac Km Curve



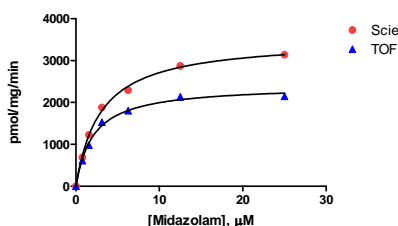
The activity of CYP2C9 was validated using Diclofenac as a probe. Using non-linear curve fitting software (GraphPad Prism), the Km and Vmax values were calculated and the Km values are within the ranges (3.4-52 μM) recommended by the FDA.

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.

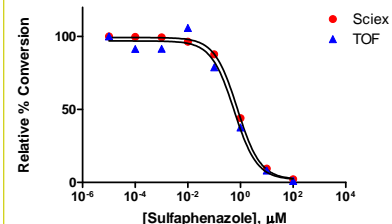
Midazolam Km Curve



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.

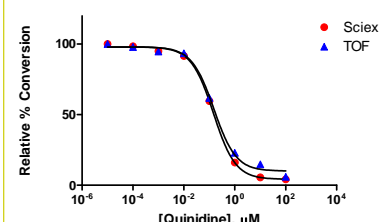
Results

Diclofenac IC50 Curve



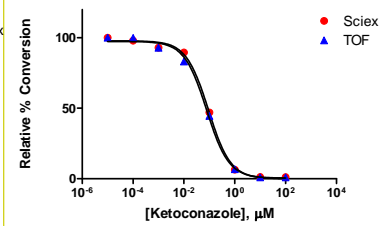
An IC50 curve for Sulfaphenazole, a known inhibitor of CYP2C9, was run using optimized conditions: 0.25mg/ml HLM, 15μM Diclofenac, 45 min incubation at 36°C, quenched with an equal volume of acetonitrile containing internal standard. The calculated IC50 values are close to values reported in literature.

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 TOF	AB Sciex	Literature*
2C9 - Diclofenac	19.79 ± 8.2	19.29 ± 0.69	3.4-52
2D6 - Dextromethorphan	5.66 ± 1.13	4.07 ± 0.65	0.44-8.5
3A4 - Midazolam	2.08 ± 0.21	2.96 ± 0.19	1-14

Measurements are within 2-fold and are consistent with literature values.

Comparison of Vmax values

	Agilent TOF	AB Sciex	Literature**
2C9 - Diclofenac (Sulfaphenazole)	5692	4651	1670
2D6 - Dextromethorphan (Quinidine)	192	176	202
3A4 - Midazolam (Ketoconazole)	2409	3513	1220

Measurements are within 2-fold and are consistent with literature values.

Comparison of IC50 values

	Agilent TOF	AB Sciex	Literature**
2C9 - Diclofenac (Sulfaphenazole)	0.57	0.76	0.27-0.75
2D6 - Dextromethorphan (Quinidine)	0.15	0.14	0.02-0.68
3A4 - Midazolam (Ketoconazole)	0.08	0.09	0.03-0.3

Measurements are within 2-fold and are consistent with literature values.

* 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

• QqQ-MS and TOF-MS gave the same results

• Km, Vmax, and IC50 values were within 2-Fold
 • Data was consistent with literature

• Accurate mass detection may provide an alternative to the time-consuming process of MRM method development associated with tandem-MS

• RapidFire in conjunction with TOF-MS may be useful for a wide range of other *in vitro* ADME assays where MS method development is a bottleneck

• Sustained throughputs of 6-8 seconds/sample