Ultra-Fast Online SPE/MS for the Efficient Screening of Cytochrome P450 Inhibition Using Cassette Analysis.

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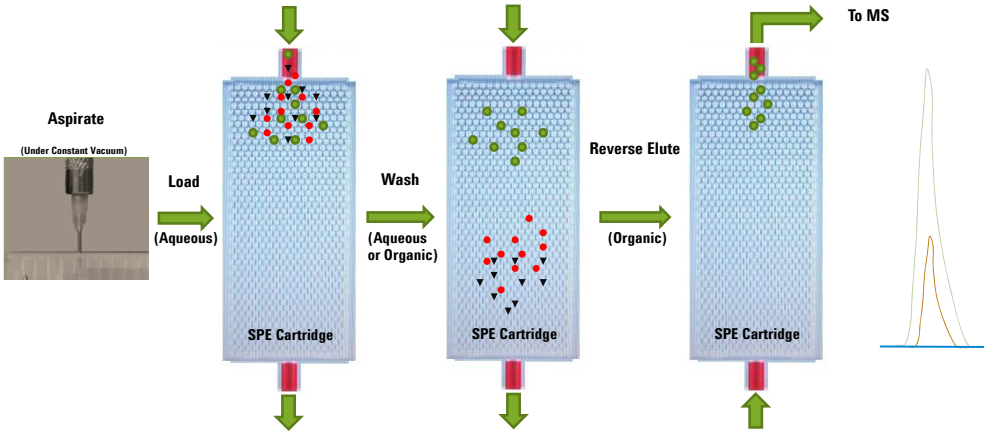
Introduction

The Cytochrome P450 (CYP) enzymes are involved in many drug metabolism transformations and account for a significant number of bioactivation and metabolic reactions. The presence of some drugs can interfere with the metabolic activity of CYP enzymes causing drug-drug interactions. Analysis of potential drug-drug interactions caused by inhibition of the enzymatic activities of CYP enzymes plays an important role in the drug discovery process. The ever increasing need to evaluate a large number of samples and the need to eliminate weaker candidates in the initial phase of the drug discovery process, has created a bottleneck at LC/MS/MS analysis. We evaluated the use of an ultra-fast online SPE/MS system (the Agilent RapidFire High-throughput Mass Spectrometry system) coupled to an Agilent QqQ and Agilent QTOF for the analysis of pooled samples across several CYP450 isoforms in a single cassette injection mode to provide an even faster and more cost-effective screen.

The RapidFire/MS System

The Agilent RapidFire High-throughput Mass Spectrometry System is an ultra-fast liquid handler that quickly solid phase extracts biological samples upstream of mass spectrometric detection. The instrument aspirates a portion of each well of a 96- or 384- well assay plate and applies the sample to an SPE column chosen to retain the analyte(s) of interest. After a washing step, the sample is reverse eluted and sent to a mass spectrometer for detection. The entire cycle time between aspiration of one well and the next is usually between 7 and 10 seconds. The RapidFire/MS system can be interfaced with an array of mass spectrometer types, depending on assay requirements.



Flowpath for RapidFire

Experimental

FDA recommended CYP drug probe substrates (CYP2C9/diclofenac; CYP2D6/dextromethorphan; CYP3A4/midazolam) were incubated individually with human liver microsomes (0.25mg/ml) and an eight point dose response curve was created with respective known inhibitors starting at 100 µM with subsequent 10X dilutions. The reaction was started by the addition of NADPH and stopped with 2X volume of acetonitrile containing respective deuterated internal standards. Generic SPE conditions were developed for all CYP450 isoforms. The analytes were solid phase extracted using a C_4 column with water: formic acid: TFA (100%:0.09%:0.01%) and eluted with acetonitrile: formic acid: TFA (100%:0.09%:0.01%). MS conditions for each compound were optimized individually for the Agilent QqQ. All the compounds were run in full scan mode within m/z range of 100 to 1700, under generic MS conditions on the QTOF.

Agilent RapidFire Settings

RapidFire Conditions	
Cycle Durations (ms)	State 1 Aspirate: 600
	State 2 Load/Wash: 3000
	State 3 Elute: 3000
	State 4 Re-equilibrate: 500
Solvents	Solvent A: H2O + 0.09% FA + 0.01% TFA
	Solvent B: MeCN+ 0.09% FA + 0.01% TFA
Column	C4

Agilent 6460 (QqQ) Settings

Source Parameters	
Ionization mode	ESI + Agilent Jet Stream
Drying gas temp.	350 °C
Drying gas flow	8 L/min
Sheath gas temp.	400 °C
Sheath gas flow	11 L/min
Nebulizer pressure	45 psi
Nozzle voltage	500 V
Capillary voltage	3500 V

Agilent 6530 (QTOF) Settings

Source Parameters	
Ionization mode	ESI + Agilent Jet Stream
Drying gas temp.	350 °C
Drying gas flow	8 L/min
Sheath gas temp.	400 °C
Sheath gas flow	11 L/min
Nebulizer pressure	45 psi
Nozzle voltage	2000 V
Capillary voltage	3500 V

Results and Discussion

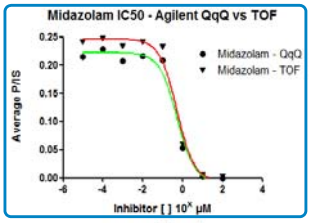


Fig.2: Individual CYP 3A4 IC_{50} curve comparison between Agilent QqQ and Agilent TOF.

Samples were analyzed on the Agilent RapidFire High-throughput Mass Spectrometry systems and strong correlations between the IC_{50} values across all isoforms were seen on both RapidFire/MS systems, as well as with values in the literature using traditional LC/MS/MS methods (corresponding IC_{50} values were within 2.0-fold of each other).

Midazolam, a CYP3A4 probe substrate, is representative of the comparison of individual IC_{50}s, on QqQ vs. TOF as shown in Fig.2.

Individual vs. Pooled IC_{50} curves for all isoforms analyzed on a QTOF Mass Spectrometer are shown in Fig. 3.

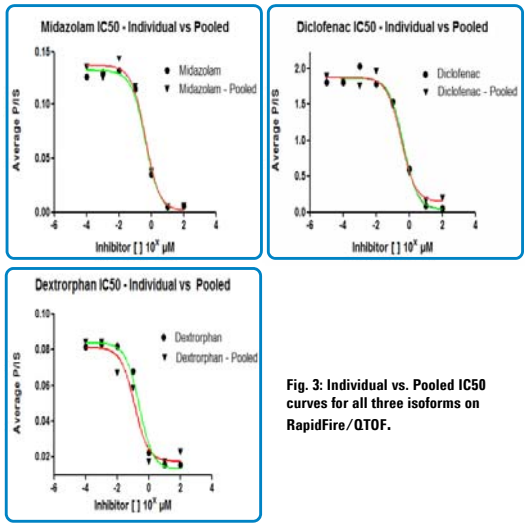


Fig. 3: Individual vs. Pooled IC50 curves for all three isoforms on RapidFire/QTOF.

Table 1: IC_{50} results for QQQ and QTOF

			IC_{50} (µM)			
			QqQ		QTOF	
Enzyme	Substrate	Inhibitor	Individual	Pooled	Individual	Pooled
CYP2C9	Diclofenac	Sulfaphenazole	0.14	0.19	0.23	0.33
CYP2D6	Dextrorphan	Quinidine	0.28	0.23	0.24	0.25
CYP3A4	Midazolam	Ketoconazole	0.50	0.51	0.49	0.44

The results in Table 1 indicate that for P450 inhibition assays, a significant increase in efficiency and throughput (>20 fold) compared to LC/MS/MS can be achieved with an analysis capacity of >350 samples per hour and no loss of integrity in the IC_{50} results.

Conclusion

- Major CYP isoforms were analyzed successfully on an ultra-fast online SPE/MS system integrated to QqQ or QTOF MS platforms.
- Significant increases in efficiency and throughput (>20 fold) as compared to LC/MS/MS can be achieved for P450 inhibition assays with an analysis capacity of >350 samples per hour.
- There was no loss of integrity in the IC_{50} results for individual as well as cassette analysis, with corresponding IC_{50} values within 2.0-fold of each other.