

# Comparison of BioTrove RapidFire uHTMS and Traditional LCMS/MS Analysis to Assess Cytochrome P450 Inhibition Utilizing Clinically Relevant Probe Substrates

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## Introduction

The capability to assess Cytochrome (CYP) 450 inhibition accurately is critical to the drug discovery process, and doing so rapidly can have a proportionally greater impact based on the ability to influence ongoing chemistry efforts. Fluorescence detection has historically been the only technology capable of high enough throughput to make this possible, but there are inherent drawbacks. Substrates for these types of assays are selected primarily for the capacity to reflect incident light, and secondarily for their suitability as substrates for particular enzymes. Recent studies have suggested that IC<sub>50</sub> values from fluorogenic probes are less reliable than was previously thought. Regulatory agencies only accept CYP450 inhibition data generated from clinically relevant probe substrates, and this has become the standard for evaluation in the industry. The throughput, however, is dependent on tandem mass spectrometry as the detection method and despite recent advances, mass spectrometry is still a major bottleneck in the process. The BioTrove uHTMS (ultra-High Throughput Mass Spectrometry) system uses a proprietary online extraction and separation technique that allows for a cycle time as low as 10 seconds per sample. With this speed a 96 well plate can be injected in about 10 minutes. Inhibition of CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 was evaluated using a variety of clinically relevant probe substrates. The substrates were incubated in the presence of inhibitors in both human liver microsomes and recombinant CYPs to compare the two matrices. Correlations between both detection methods were determined to assess the suitability of each of the enzyme/substrate pairs.

## Materials and Methods

A total of sixteen AstraZeneca NCE's were incubated with recombinant CYP450 enzymes (BDGentest) and human liver microsomes (BDGentest, AstraZeneca Global batch) under the conditions listed below. The reaction conditions for the microsomal incubations are the same as shown below, except that a microsomal concentration of 0.2 mg/mL is used instead of an enzyme concentration.

Table 1: Recombinant CYP (and microsomal) IC<sub>50</sub> assay conditions

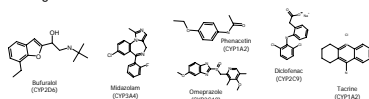
Enzyme	Enzyme Conc (nM)	Substrate	Substrate Conc (µM)	Incubation Time (min)	Km (µM)
CYP1A2	20	Phenacetin	20	15	20
CYP1A2	10	Tacrine	14	10	14
CYP2C9	20	Diclofenac	5	15	3
CYP2C19	20	Omeprazole	20	15	20
CYP2D6	20	Bufuralol	5	10	5
CYP3A4	20	Midazolam	3	10	3

The reactions were initiated by the addition of NADPH, and were stopped by addition of acetonitrile w/0.1% formic acid with an internal standard. The samples were centrifuged at 4000 rpm for 15 minutes at 4°C, and divided into two separate plates. One set of plates was sent to BioTrove for HTMS analysis, and the other was retained for LCMS/MS analysis on a Sciex API4000. IC<sub>50</sub> curves were generated and the data were loaded into SpotFire for correlation analysis.

Figure 1: BioTrove UHTMS System



Figure 2: LCMS Probe Substrates



## Results

Figure 3 shows the IC<sub>50</sub> correlations for recombinant CYP450 3A4, 2C9, 2D6, 2C19, and 1A2 analyzed on a Sciex API4000 and the BioTrove HTMS system. Figure 4 displays the IC<sub>50</sub> correlations for the same CYP450 enzymes utilizing human liver microsomal fractions rather than recombinant enzymes. CYP2C19 was not included in the microsomal correlation analysis because the probe substrate, omeprazole, is not amenable to HTMS analysis due to the presence of multiple hydroxylation products. The evaluation of CYP1A2 in microsomes was also not included because analysis of phenacetin on the HTMS failed due to in-source fragmentation.

Figure 3: pIC<sub>50</sub> correlations using recombinant CYP450 enzymes

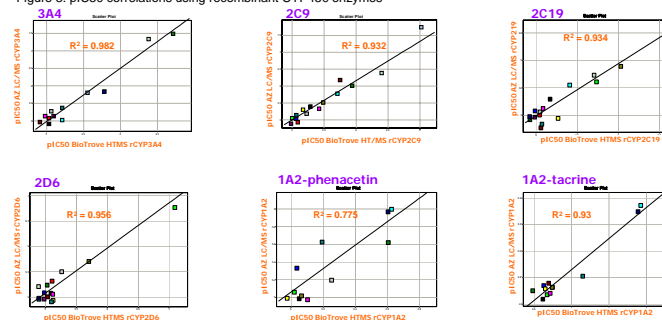
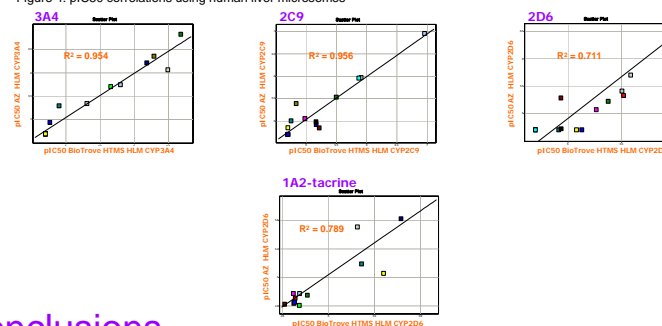


Figure 4: pIC<sub>50</sub> correlations using human liver microsomes



## Conclusions

The results from this validation study support the use of the BioTrove HTMS system as an analytical platform for LCMS CYP inhibition studies involving CYP3A4, CYP2D6, CYP2C9, and CYP2C19 for recombinant studies and CYP3A4, CYP2C9, and CYP2D6 for microsomal studies. The CYP1A2 substrate, phenacetin, appears to be unsuitable for use with this system. A second substrate, tacrine, is shown to correlate more closely between the two analytical systems and is the preferred substrate for future CYP1A2 incubations, both with recombinant and microsomal protein. The CYP2D6 substrate bufuralol may not be appropriate for microsomal incubations, and future work will determine appropriate substrates for CYP2D6 and CYP2C19 for microsomal incubations.