

Technical Note

CYP Inhibition Assays: Versatility in the ADDA High Speed Dual Arm Autosampling System

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Introduction

CYP450 inhibition studies are an important component of pharmaceutical drug discovery testing. They are most often used to determine the potential for drug-drug interactions (DDI's). In early stage work the DDI-screen is often run at a single inhibitor concentration to increase throughput. The single-point IC_{50} format can help develop an SAR within a structural class, and provides guidance for more definitive work when needed. In later stage drug discovery, multi-point inhibitor studies are employed to determine a definitive IC_{50} for the given drug molecule. Often these studies are performed in "cocktail" format, wherein a group of 5-8 CYP isoform specific substrates are tested together against a single drug inhibitor compound. A multi-point cocktail-DDI study provides a complete CYP inhibition profile of the drug candidate.

We used the multi-point cocktail DDI assay to highlight three LC methodologies available with the ADDA system. High-speed "trap and elute" LC (10-sec/sample) was compared with "single-arm" (140 sec cycle time, conventional gradient) and "dual-arm" gradient modes (90 sec cycle time, conventional gradient, dual eluent stream).

The gradient approach is often considered more definitive; it was of interest to compare results across the three methodologies, run back to back, using the ADDA system.

Five CYP 450 isoforms (1A2, 2C8, 2C9, 2D6, 3A4) and one control inhibitor (Miconazole) were tested with three setups and compared on a single ADDA system (the setups, as represented in the ADDA software, are mapped in figures below):

ADDA System Features:

High Speed

- 2 High Speed Robotic Arms
- Highest throughput for traditional LC/MS Systems

Random Access

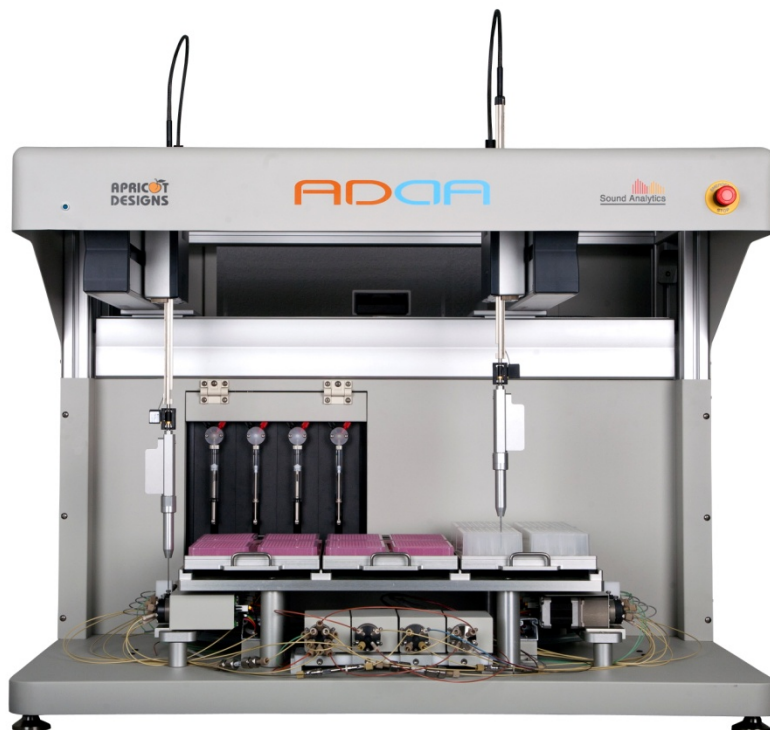
- Minimal reformatting

Flexibility

- 8 Injection Ports for independent plumbing setups

Quality

- Support for both High Speed *in vitro* and High Quality *in vivo* gradient chromatography



Injection to Injection Times

Setup 1 (Trap and Elute):	10 Seconds
Setup 2 (Dual Arm Gradient):	90 Seconds
Setup 3 (Single Arm):	140 Seconds

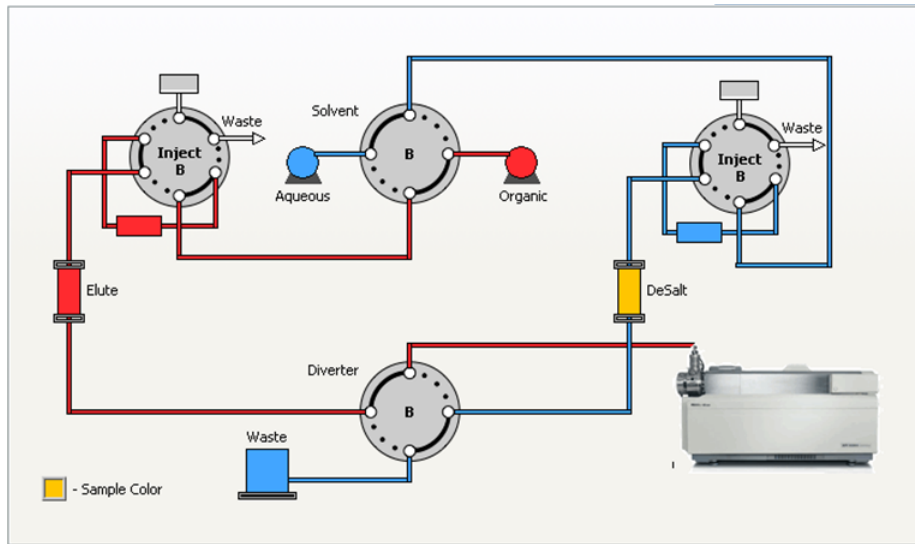


Figure 1. Dual Arm Trap and Elute Plumbing (Setup 1), as represented in ADDA Software.

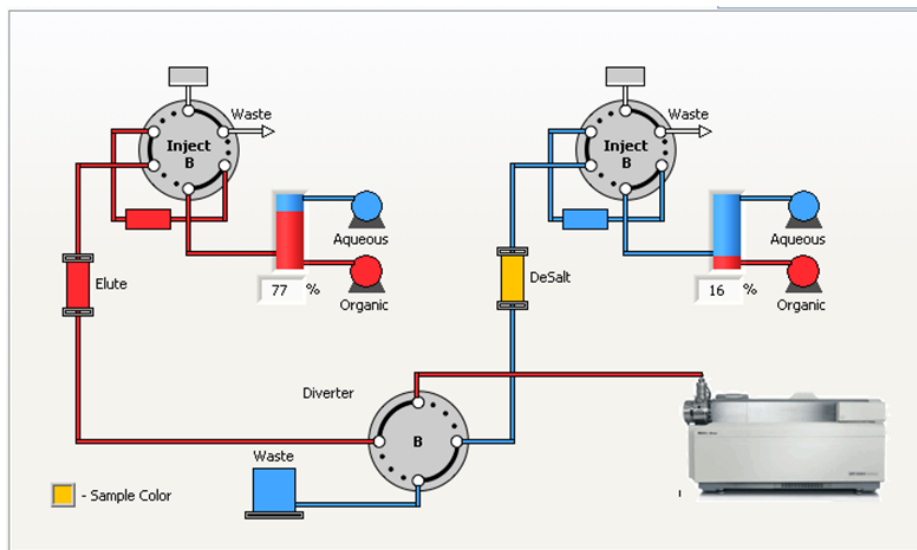


Figure 2. Dual Arm Gradient plumbing (Setup 2), as represented in ADDA Software. The single-arm gradient mode uses the same plumbing to process a single stream on one side of the instrument.

Method Details

Samples were prepared in 384 well plate incubations, with 8 inhibitor concentrations in triplicate.

Inhibitor Concentrations (uM): 30 ▪ 10 ▪ 3 ▪ 1 ▪ 0.3 ▪ 0.1 ▪ 0.03 ▪ 0.01

Compounds per plate: 16

CYPs Measured: 1A2 ▪ 2C8 ▪ 2C9 ▪ 2D6 ▪ 3A4

Control Inhibitor: Miconazole

Mass Spectrometer: AB SCIEX 5500 Triple Quadrupole

LC Conditions

	(1) Trap/Elute	(2 & 3) Gradient Runs
Column	Luna Phenyl-Hexyl 2X10 mm 5 μ	Kinetex 50X2.1mm 2.6 μ
Flow Rate	1.8 mL/min	250 μ L/min
Mobile Phase	0.1% formic acid/ acetonitrile	0.1% formic acid/ acetonitrile
Other	Equil/desalt: 4sec/5sec	Gradient: 15%B to 80%B

Marker Substrate metabolite transitions

Metabolite	CYP450	MRM
1-OH-tacrine	1A2	214.9>182.1
6a-hydroxypaclitaxel	2C8	870.5>525.2
4-OH-diclofenac	2C9	312.2>230.2
Dextrorphan	2D6	258.3>157.0
1-OH-midazolam	3A4	342.3>203.1

Results

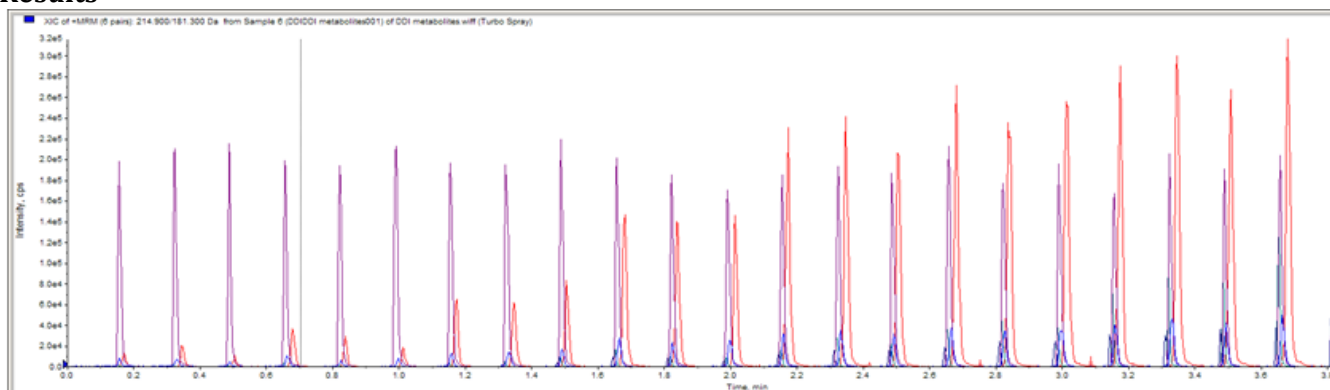


Figure 3. 10 second injections in dual arm trap and elute setup, 24 cocktail DDI samples in 4 minutes.

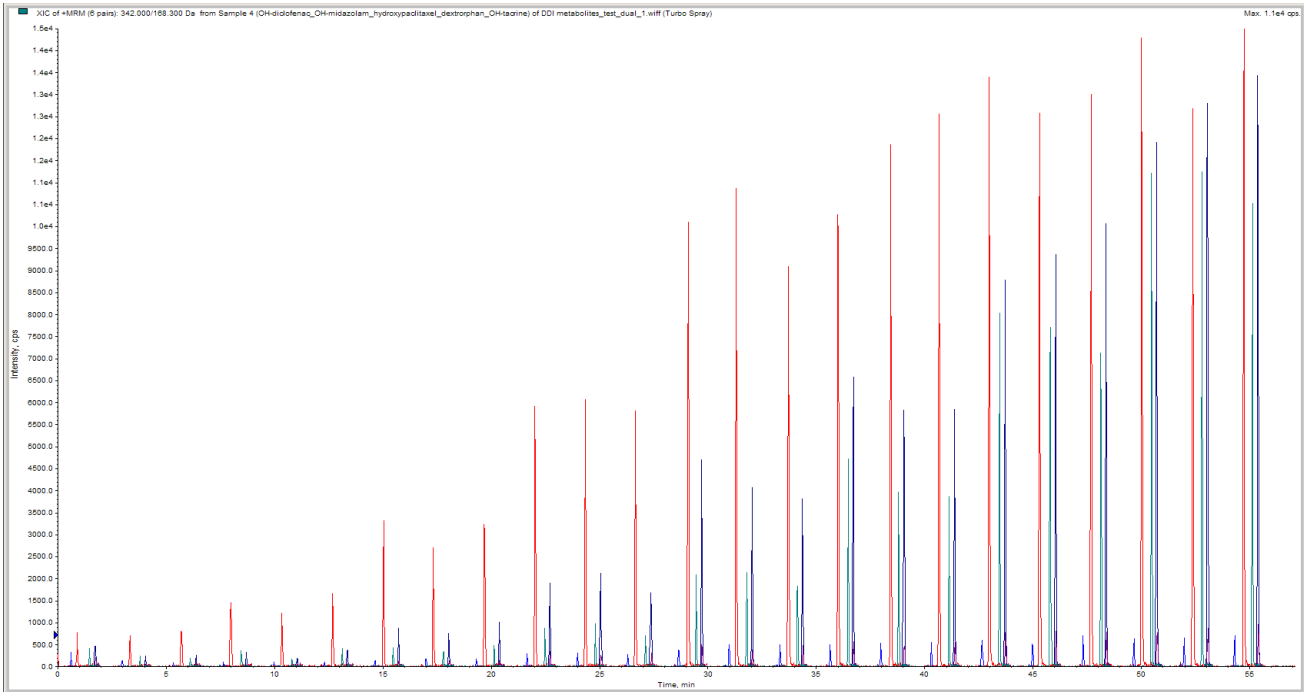


Figure 4. Single-arm gradient mode, 140 sec gradient cycle time, 24 cocktail DDI samples in ~56 min

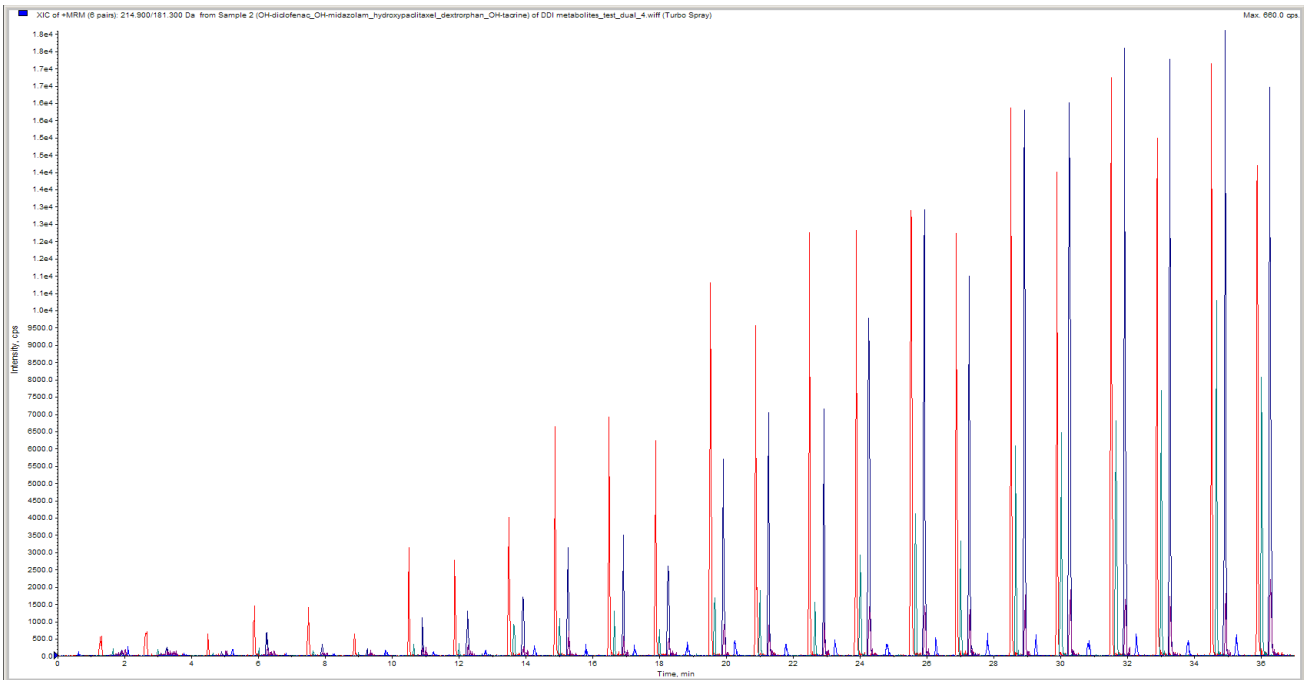


Fig 5. Dual-Arm gradient mode, 90 sec cycle time, 24 cocktail DDI samples in ~36 min

All three analyses returned excellent data. The IC₅₀ curve for CYP2C9 using both miconazole and sulfaphenazole is shown in Figure 6. For one complete IC₅₀ run on a single test inhibitor [i.e. 8-inhibitor concentrations, in triplicate (24 samples)], the trap and elute approach took 4 min, the dual-arm gradient 36 min and single-arm gradient 56 min, respectively.

DDI Cocktail IC₅₀ Assay for CYP2C9 Using Various MS Methods with Miconazole and Sulfaphenazole as the Inhibitor

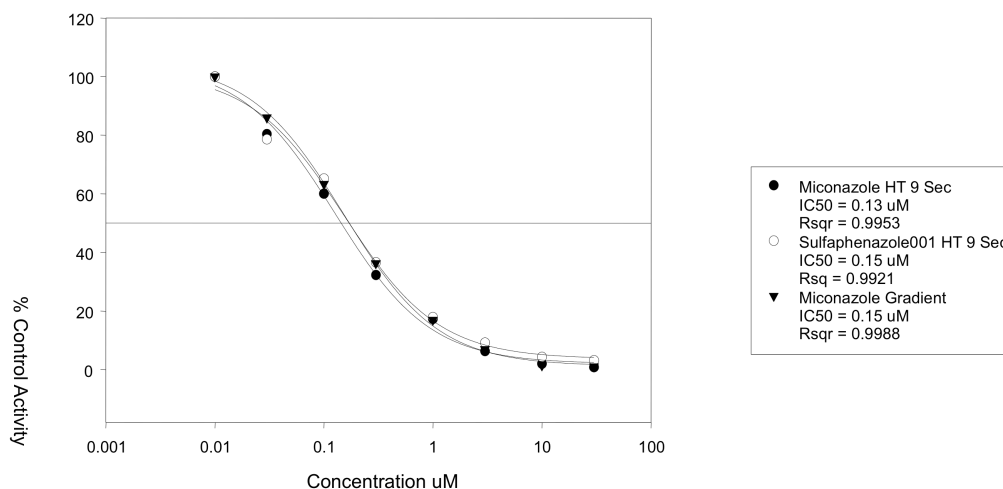


Figure 6. IC₅₀ curves for the 3 types of analysis, confirming the correctness of the 10s trap and elute result.

Conclusions

Technologies designed to increase throughput have taken two forms: parallel chromatography approaches that involve complex valving and multiple eluent streams, and faster robotics systems that decrease injection-to-injection cycle time. These latter systems cannot easily perform conventional gradient chromatography.

The ADDA system serves as a single instrument that can perform both very high-throughput (10s per sample in the case presented above) and full gradient with robustness and quality. The equivalence of the IC₅₀ curves in this example gives a strong indication that fast trap and elute methods can be used even on equipment that gives the full range of chromatographic options. Simple software setup and multiple injection ports allow for these options to be plumbed on the system simultaneously, giving the versatility needed to perform different types of analyses automatically and/or remotely in a queued set of batches on the ADDA system.

