

OVERVIEW

Purpose

- Label-free High Throughput screening of CYP mediated metabolism

Method

- Acoustic deposition of compound
- Assay incubation and quench
- Acoustic deposition to analysis plate
- LDTD-MS/MS quantitative analysis in 1.1 seconds sample to sample
- Data analysis with MultiQuant™ software

INTRODUCTION

Screening drug candidates for potential liabilities, such as drug-drug interactions (DDI), is of utmost importance in drug discovery. Inhibition of CYP-mediated metabolism can cause DDI in humans by increasing or decreasing the efficiency of another drug, or preventing the formation of an effective metabolite. Label-free screening using physiologically relevant substrates has increased the demand for mass spectrometry (MS) giving better specificity and less false positives. Improving MS throughput, in a true high-throughput screening environment, remains a challenge for conventional ionization sources.

Advances have been shown (Journal of Biomolecular Screening - 2015) using a new high throughput ion source (LDTD) that enables an analysis time of < 1.9 seconds sample to sample (per well). Cross validation was carried out in a 384-well format with RapidFire™-MS/MS which has a readout time of ~10 seconds per sample. The Laser Diode Thermal Desorption ion source (LDTD) interfaced with tandem MS was coupled with acoustic sample deposition using samples of 100 nL deposited onto an LDTD-special stainless-steel 384-LazWell plate. Samples are desorbed directly from the plate's surface into gas-phase using thermal desorption by an infrared laser, ionized by APCI and analyzed by MS/MS.

LDTD® Ionization Source:

The LDTD® uses a Laser Diode to produce and control heat on the sample support (**Figure 1**) which is a 96- or 384-well plate. The energy is then transferred through the sample holder to the dry sample which vaporizes prior to being carried by a gas in a corona discharge region. High efficiency protonation and strong resistance to ionic suppression characterize this type of ionization, and is the result of the absence of solvent and mobile phase. This allows for very high throughput capabilities of 1.1 seconds sample-to-sample analysis time, without carry over.

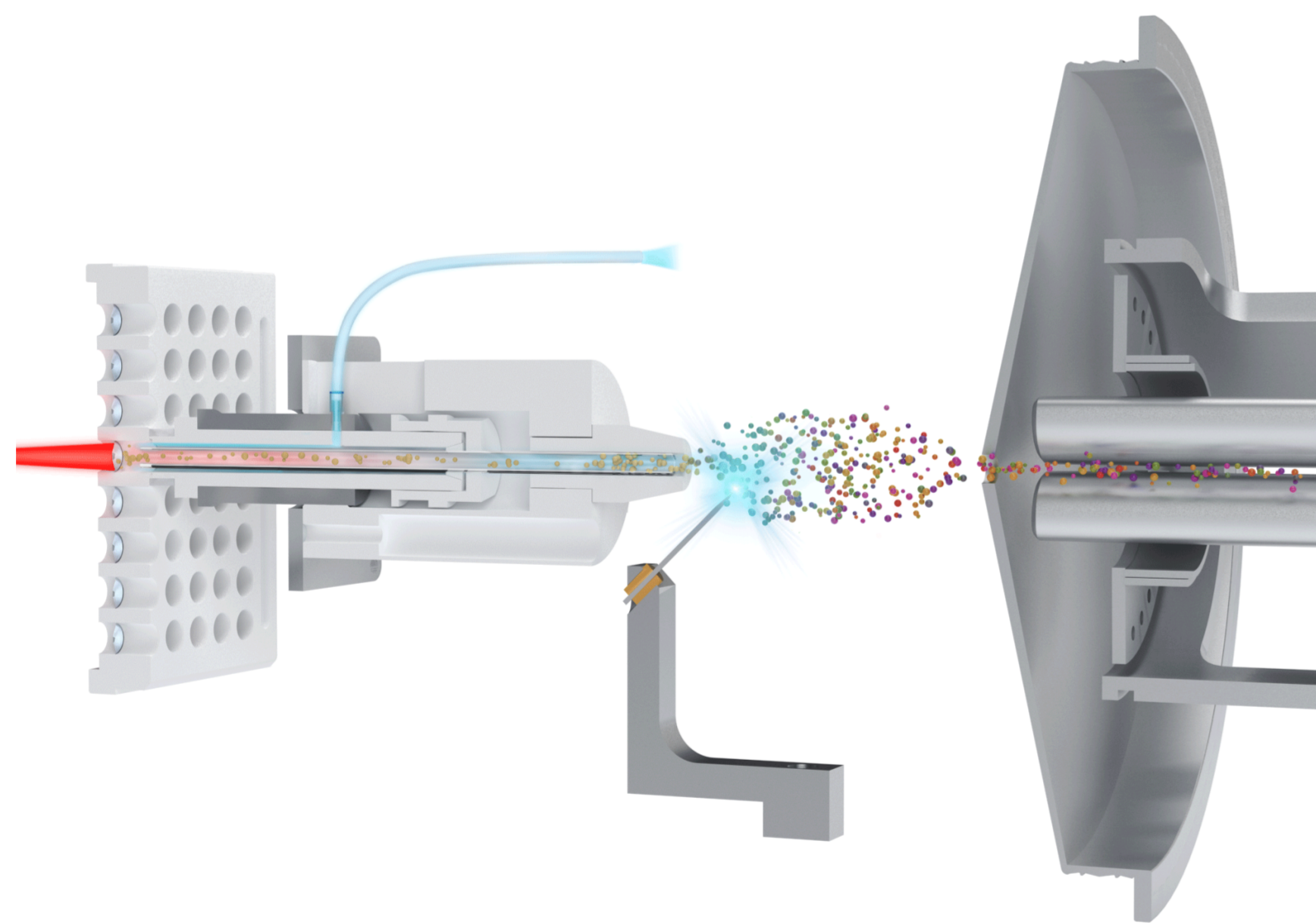


Figure 1 Schematic of the LDTD ionization source

METHOD

Sample preparation:

Reaction Component/Step	Vol / well; Time	Instrument
Test Samples	40 nL	ECHO 550
Background Buffer	10 µL columns 23-24	Multidrop Combi
Enzyme Buffer (warm)	10 µL whole plate	Multidrop Combi
Pre-incubation Time Course	30 min	37°C
Substrate Buffer (room T°)	10 µL columns 1-22	Multidrop Combi
Probe reaction time	CYP2D6: 7 min CYP2C9: 10 min	37°C
Quench/IS Buffer	20 µL	Multidrop Combi
Process	Dispense 100 nL onto LazWell plate	ECHO 550

Mass spectrometer settings:**MS Parameters**

- APCI (+)
- Dwell: 2 msec
- Corona discharge: 3 µA
- DP: 100 V
- Multiple Reaction Monitoring (MRM)

LDTD Parameters

- Laser power pattern :
 - Increase laser power to 100 % in 0.1 sec
 - Maintain for 0.2 sec
- Carrier gas flow (Air) : 3 L/min
- Model S-3840 with plate stacker

WORKFLOW

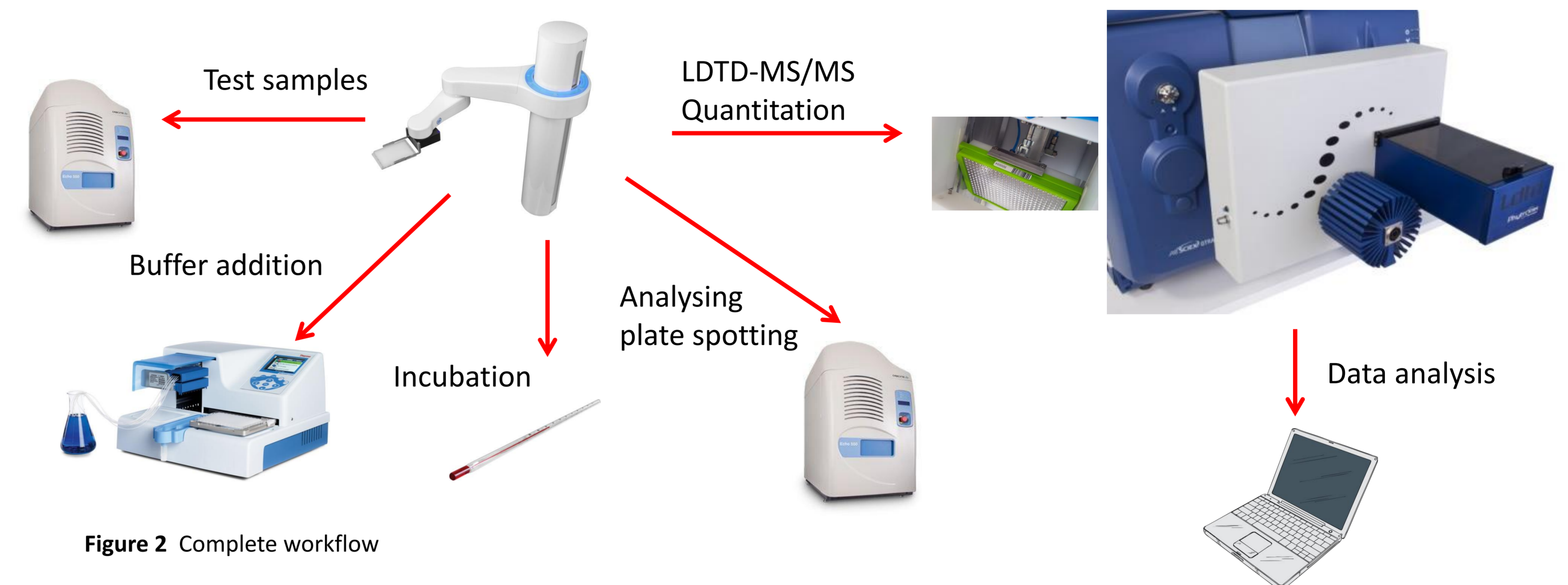


Figure 2 Complete workflow

RESULTS

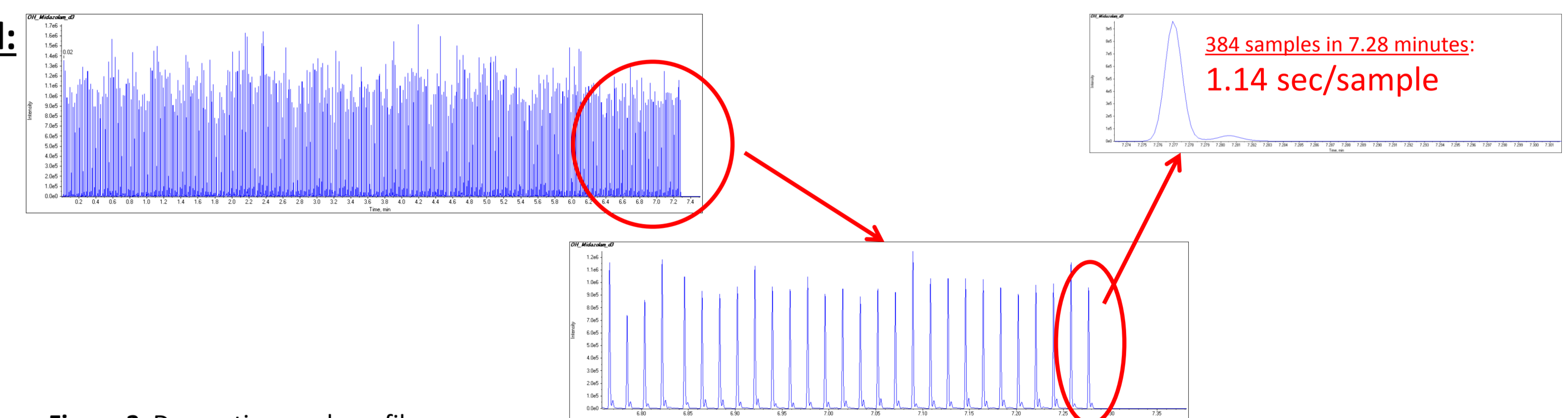
Analysis speed:

Figure 3 Desorption peak profile

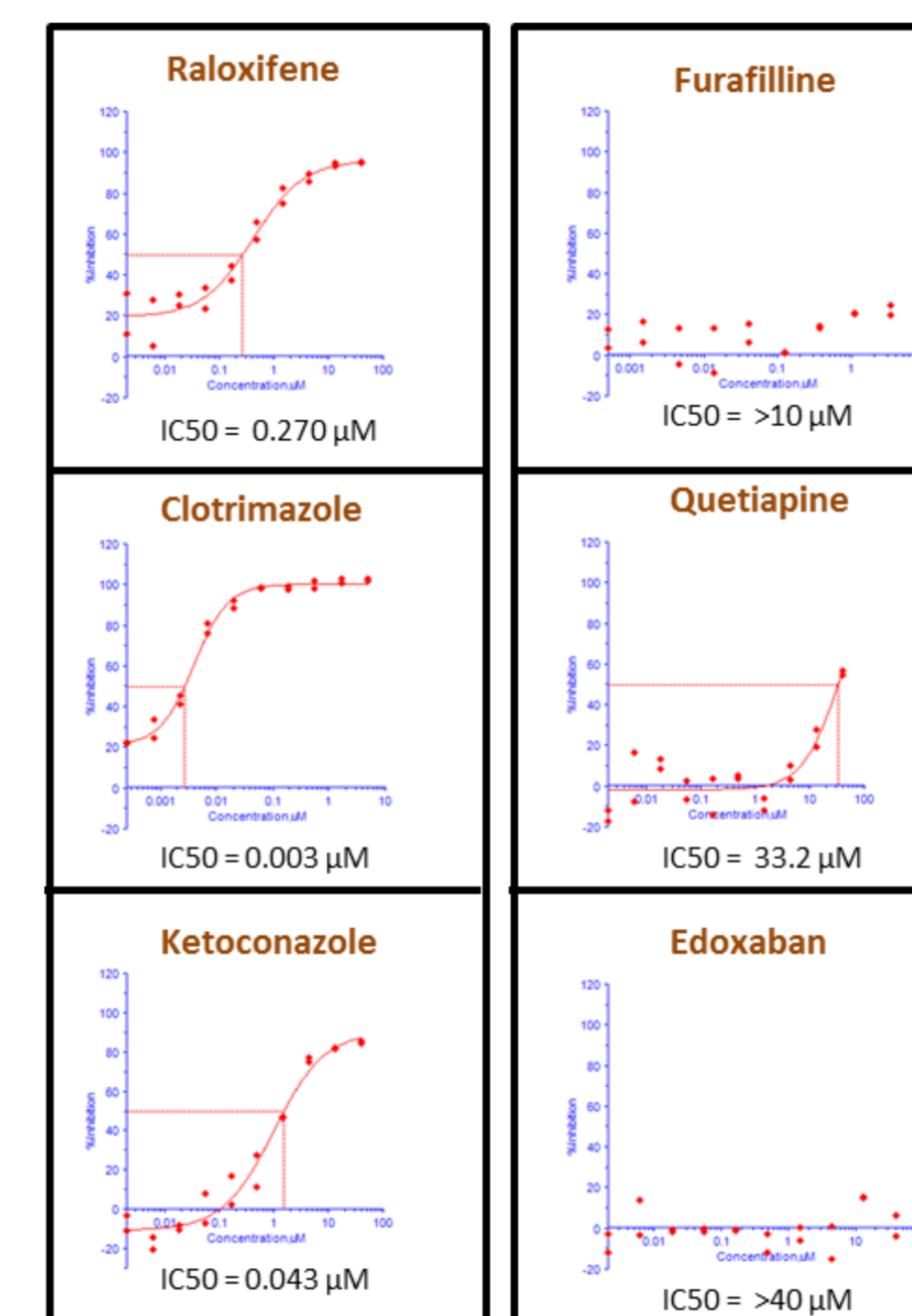
IC50 determination:

Figure 4 Positive and negative control for CYP 3A4

IC50 curve:

Enlarged view of the LDTD-MS/MS intensity vs time plot showing acquisition of CYP3A4 analyte (1'-OH-midazolam) in blue and IS ([13C3]1'-OH-midazolam) in pink

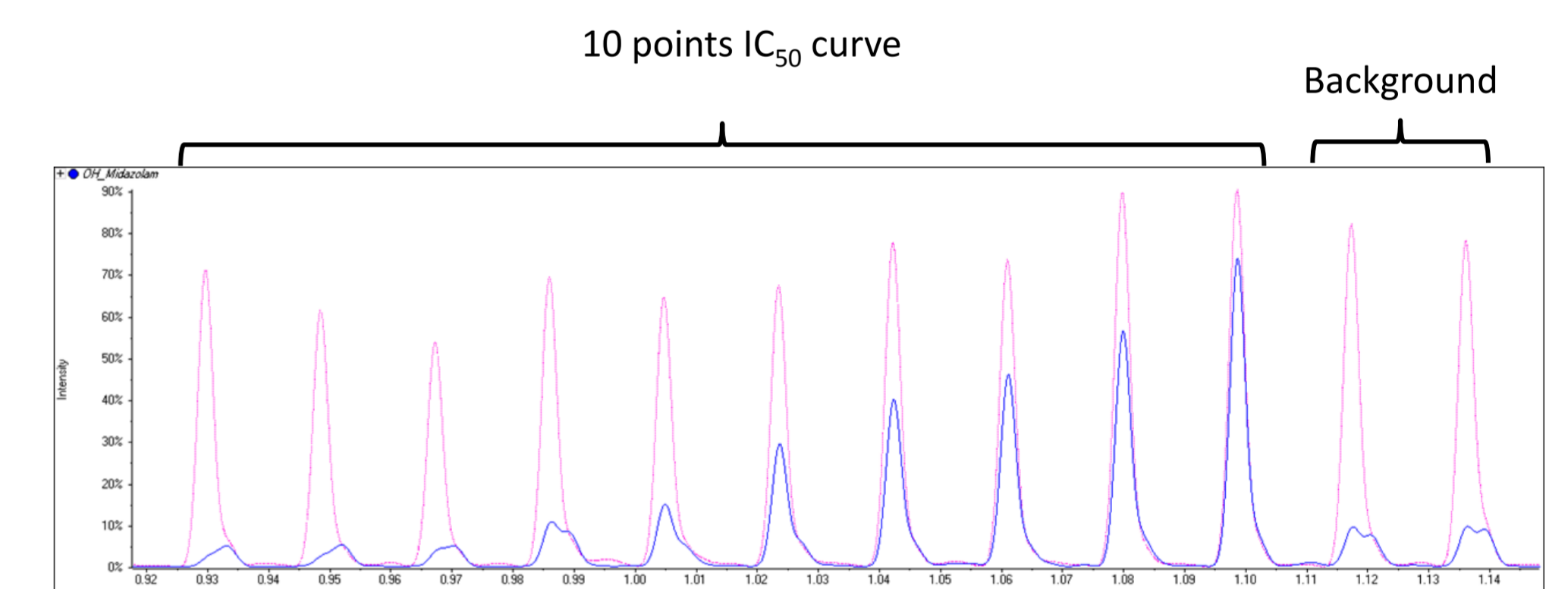


Figure 5 Desorption peak profile (zoom)

Discussion:

- IC50 determination of CYP mediated metabolism remains accurate when increasing speed by 39% compared to the reference method¹ that was cross validated.
- LDTD new plate stacker entry allows continuous mass spectrometric measurement.
- Mass spectrometer operation requires minimal dwell time acquisition to reach sufficient data points for adequate peak definition.

¹ Haarhoff et Al., "Coupling Laser Diode Thermal Desorption with Acoustic Sample Deposition to Improve Throughput of Mass Spectrometry-Based Screening", Journal of Biomolecular Screening, 2016, Vol 21 (2) 165-175

CONCLUSION

- Label-free High Throughput screening of CYP mediated metabolism
- Accurate IC50 determination
- Throughput of **1 sample every 1.14 seconds**
- Continuous operation with robotic arm and plate stacker
- Example of 12 plates readout in less than 1.5 hours
- Possibility of multiplexing¹ assay deposition onto the same analysis plate to increase throughput by a factor of 3 to 6 (0.38 to 0.19 sec/sample)