

High-Throughput LDTD384-MS/MS for Drug Metabolism and Pharmacokinetic Studies

¹Sébastien Gagné; ²Patrice Tremblay; ¹Francis Foczeny; ¹Robert Houle;
¹Eric Langlois; ¹Kevin Bateman; ²Pierre Picard

¹*Merck Canada, Kirkland, QC, Canada*

²*Phytronix Technologies, Quebec, QC, Canada*

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Background

- Drug discovery
 - Process by which potential drugs are discovered through new chemical entities (NCE)
 - Involve several fields
 - One of them → Drug Metabolism and Pharmacokinetics (DMPK)
- DMPK criteria (assessed using mass spectrometry)
 - Pharmacokinetics
 - Drug-Drug Interaction
 - Metabolic stability
 - Metabolic profiles (in vitro / in vivo)
 - Distribution and route of elimination
 - Plasma Protein Binding
 - Permeability
 - Transporter
 - Blood / Plasma
 - PK prediction
 - Phenotyping

Can we differentiate compounds faster?

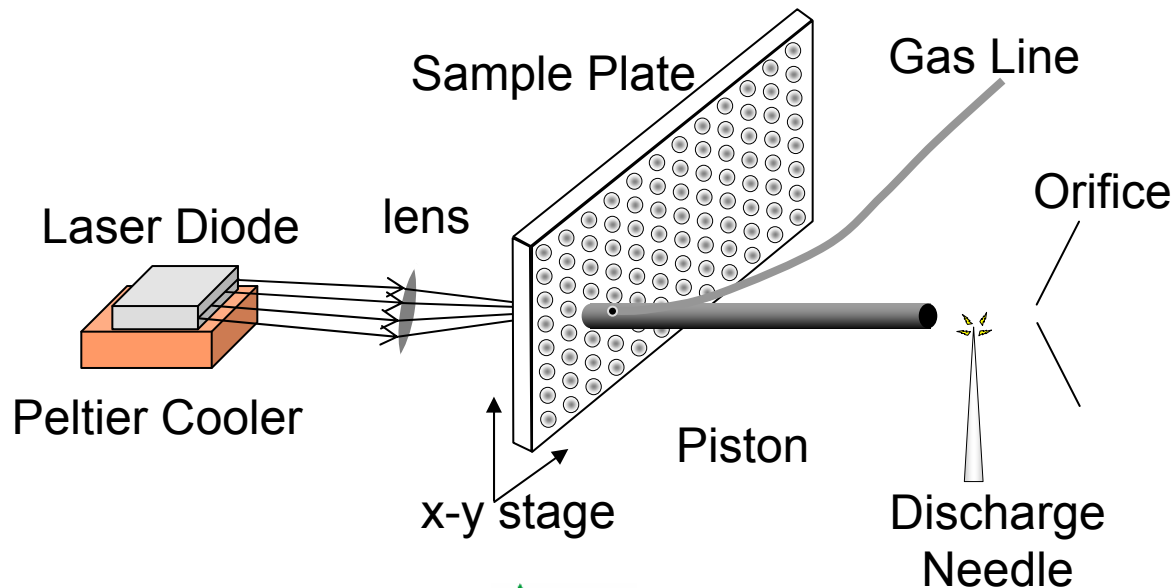
Introduction

- The LDTD source

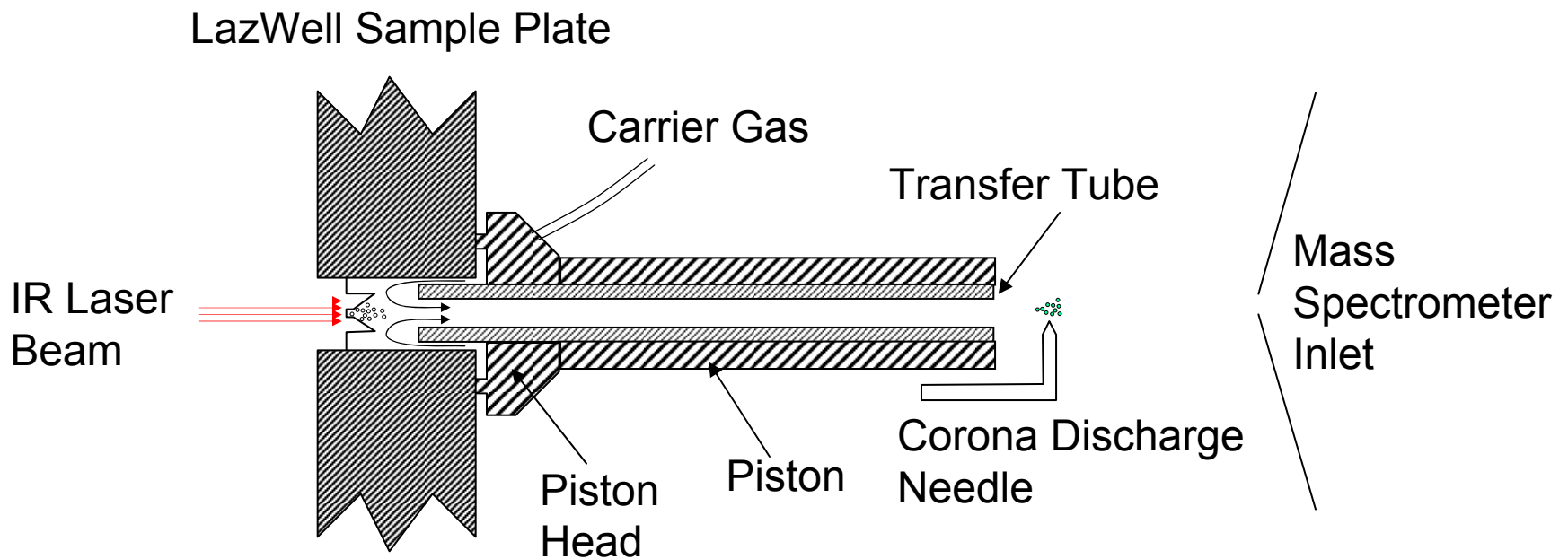
- Developed by Phytronix Technologies (Québec, QC, Canada)
- Indirect thermal desorption by a laser diode (980 nm, 20 W)
- Atmospheric pressure chemical ionization (APCI)
- Atmospheric pressure, fit with any mass spectrometer
- Minimal sample prep, no LC separation
- No solvent, no matrix application



Laser diode



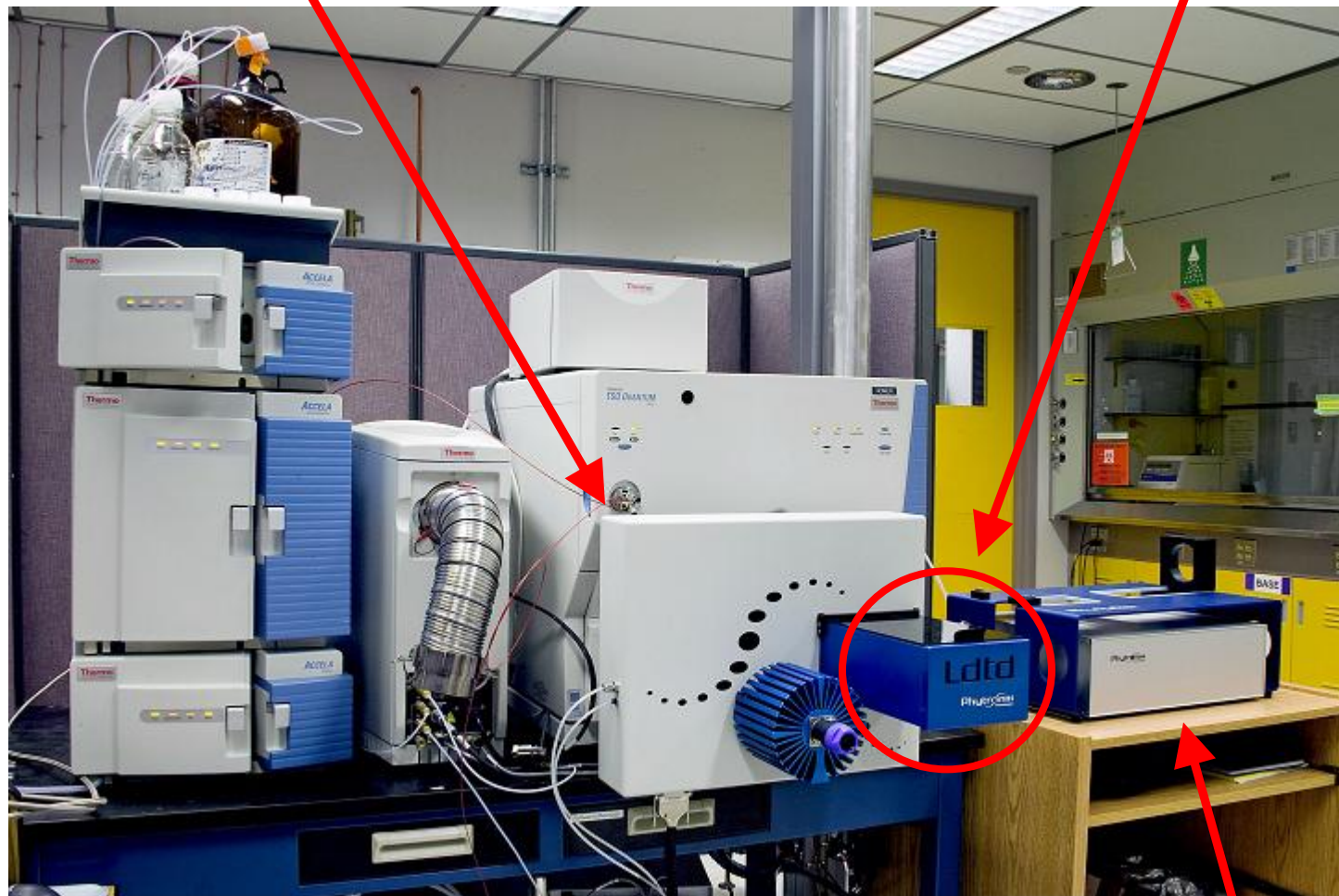
Schematic of the LDTD Source



LDTD Source on Thermo-Quantum

LDTD source

10-plate sample loader



Source power supply
& gas controller

Experimental details

• Instrumentation

- LDTD source + Thermo Quantum
- LazWell sample plate
 - **NEW Standard 384-well plate format**, disposable
 - Well: proprietary stainless steel alloy
 - 1-2.5 μ L of sample per well (**no matrix**)
 - **No carryover**: each sample has its own well
 - Amenable to robotic sample preparation systems
 - Current well design: hexagonal



LazWell plate

• Experimental condition set up

- The SRM conditions of each analyte/internal standard were optimized directly by LDTD-MS using the Quantum Tune page (positive mode)
 - 1 μ L of each standard was spotted on the LazWell plate, dried, and laser power/duration, carrier gas and SRM conditions were optimized individually.
- Carrier gas for LDTD: compressed air, 35°C, 3 L/min

LDTD384-MS/MS – Over all performances

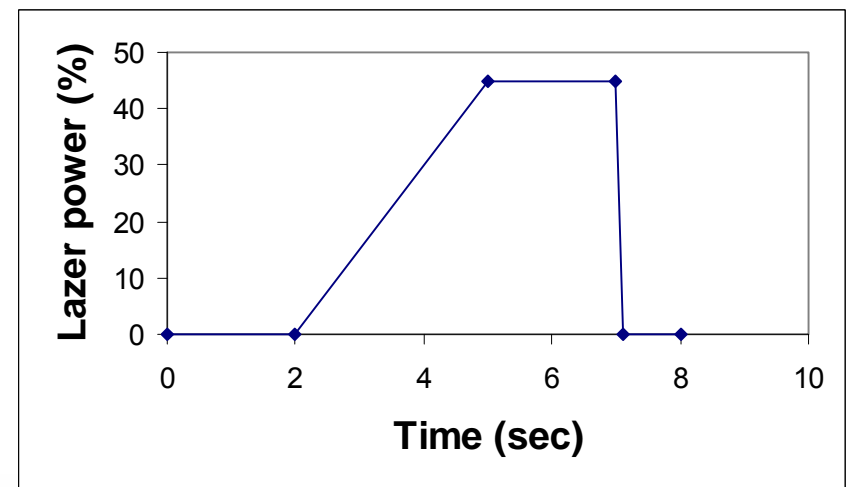
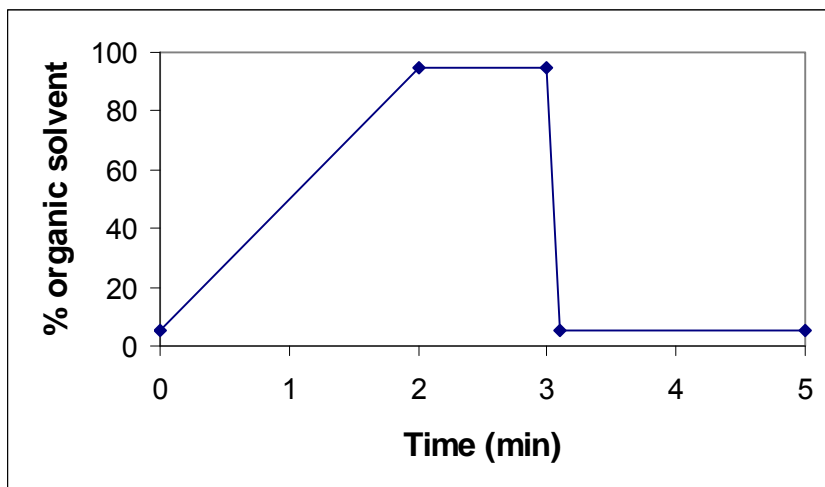
**HPLC-MS/MS
(API4000)**

**Run time =
5 minutes (300 seconds)
sample-to-sample**

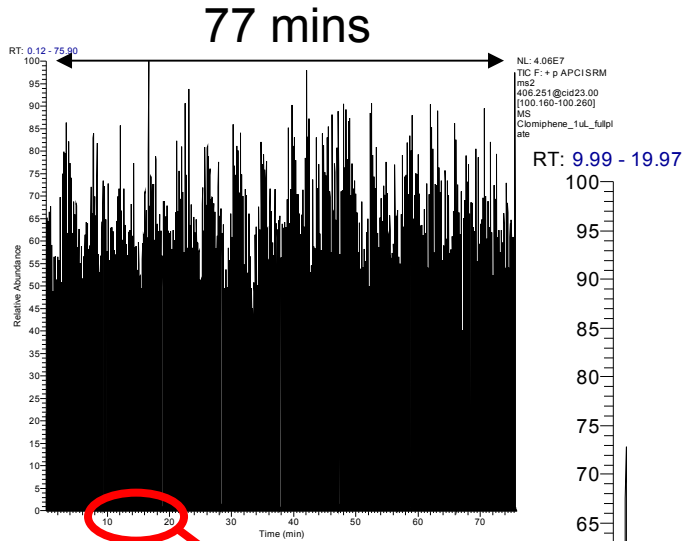
**LDTD384-MS/MS
(Quantum)**

**Run time =
12 secondes
sample-to-sample**

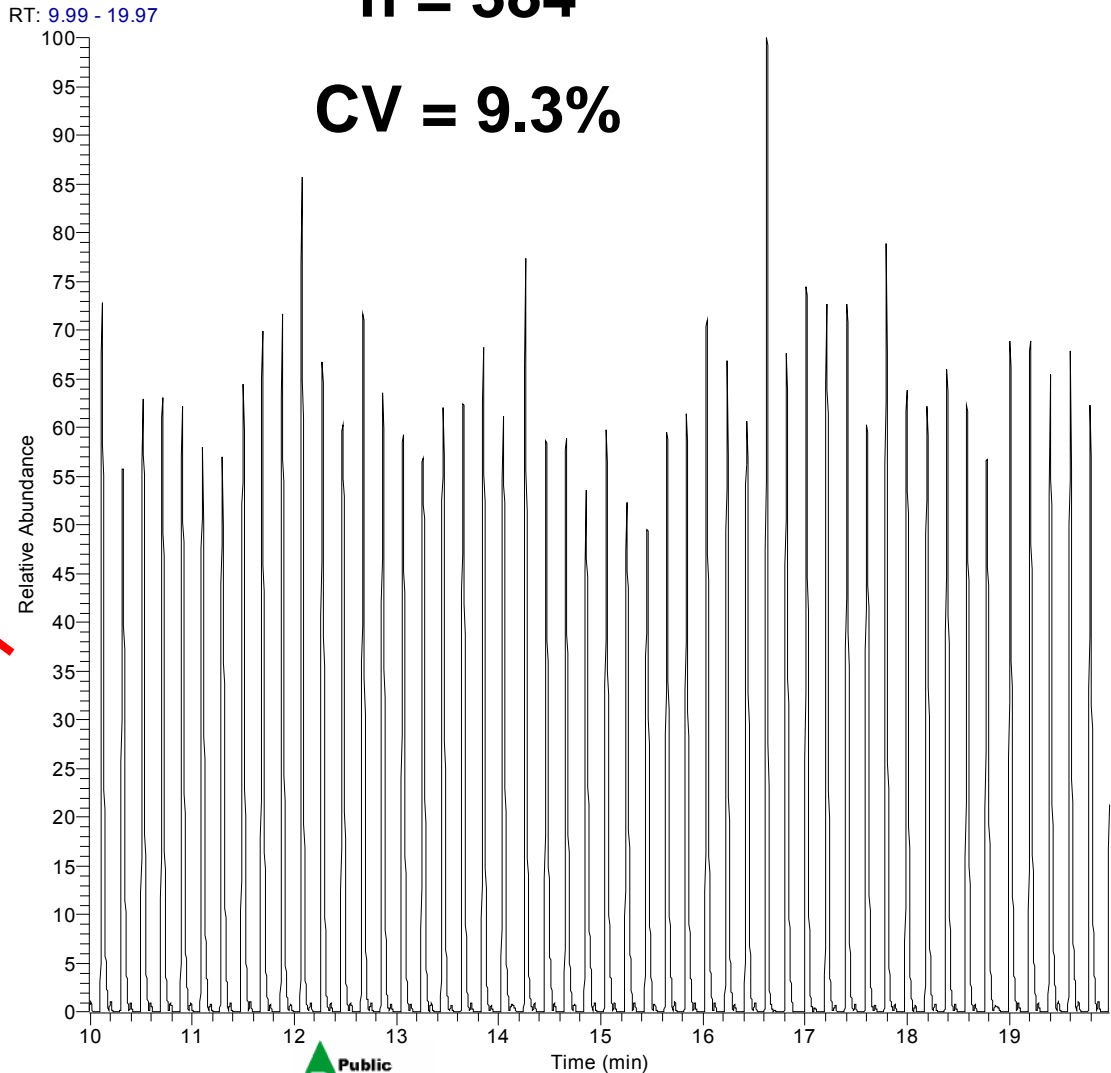
25x faster



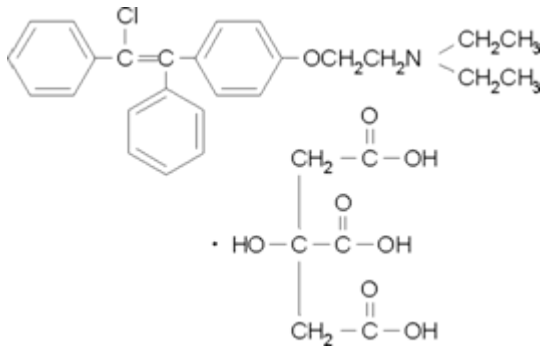
LDTD384-MS/MS – Over all performances



n = 384
CV = 9.3%



Clomiphene (1 μ M)

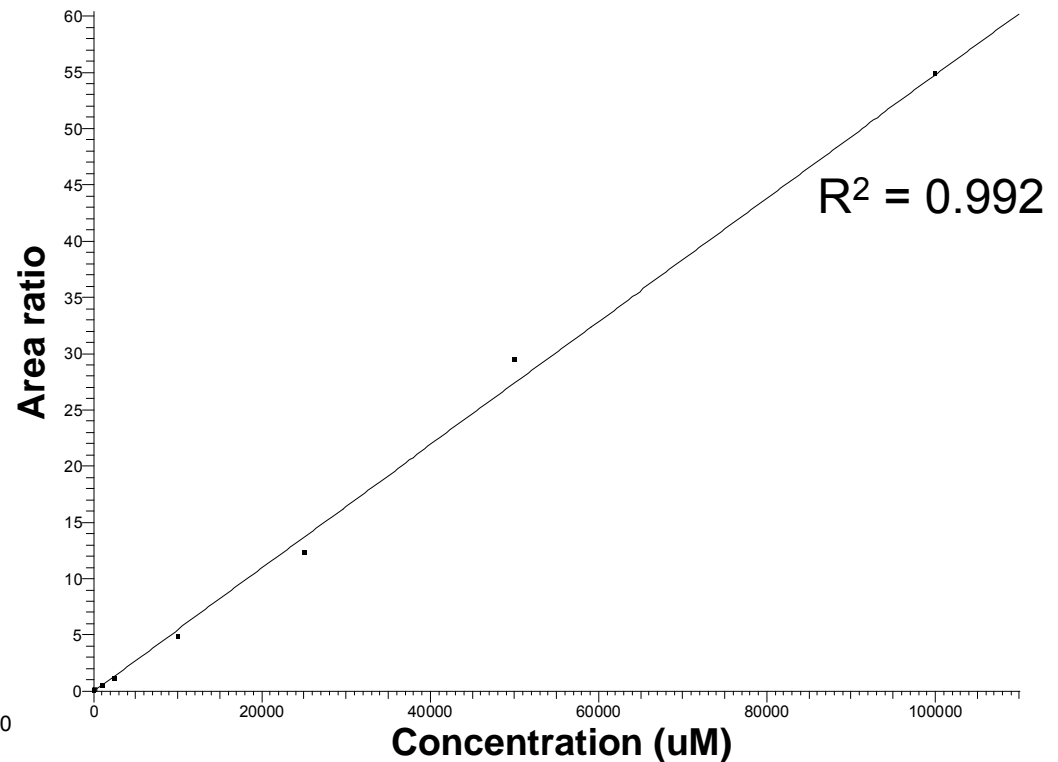
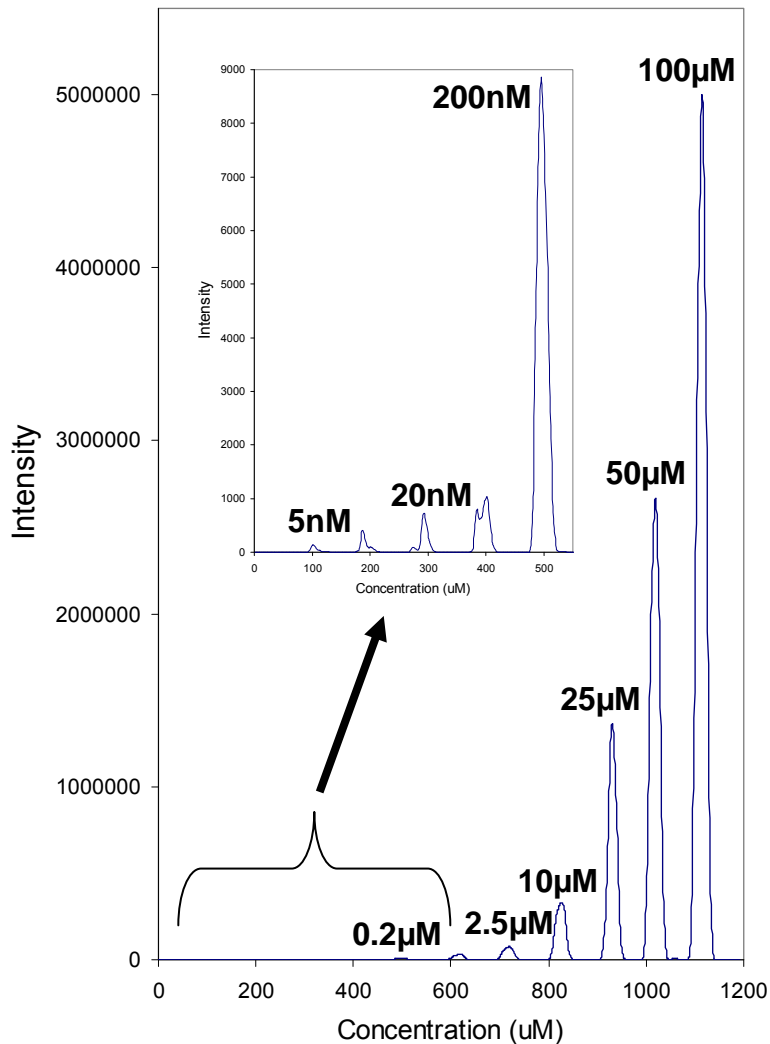


LDTD384-MS/MS – Over all performances

- Plasma samples spiked and quenched with 3 volumes of ACN and diluted with 5 volume of MeOH + EDTA

- LOD ~ 5nM

- 5 order of magnitude

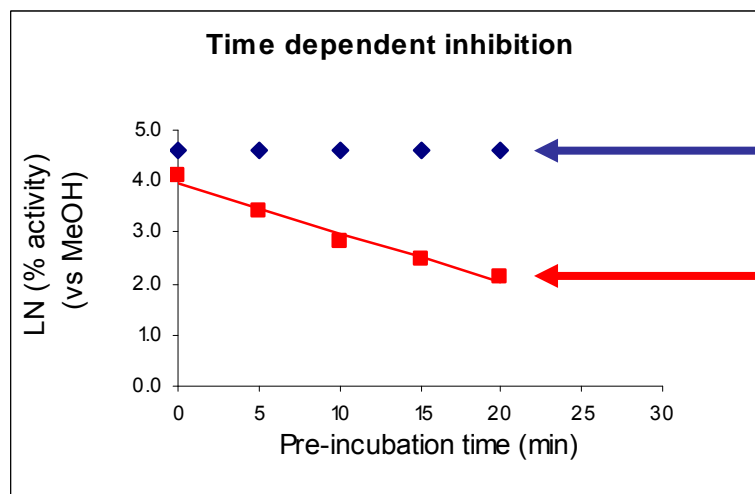
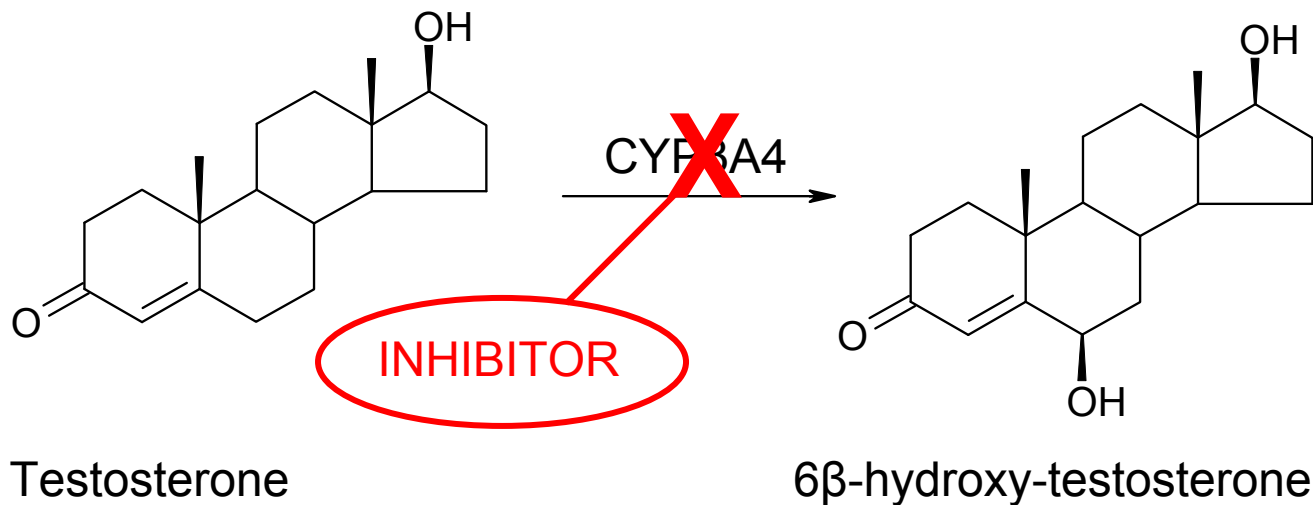


Applications

- Application #1: Drug-drug interaction
 - Time-Dependent Inhibition
 - Ability of a drug to decrease CYP activities
- Application #2: Metabolic Stability/Intrinsic Clearance
 - In vitro tool used to predict the stability of a compound
- Application #3: Pharmacokinetics
 - Explore what the body does to the drug

Application #1 – Time Dependent Inhibition

- K_{obs} → Test compound at 10uM & 50uM } Screening approach
- Pre-incubation: 0', 5', 10', 15', 20' }

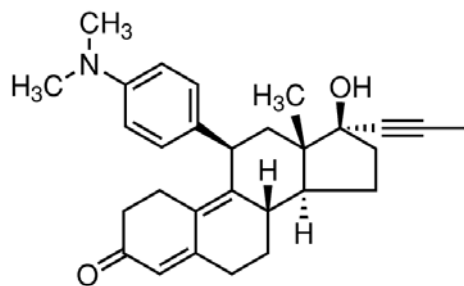


No TDI

TDI if slope
 $k > 0.015\text{min}^{-1}$



Application #1 – Time Dependent Inhibition



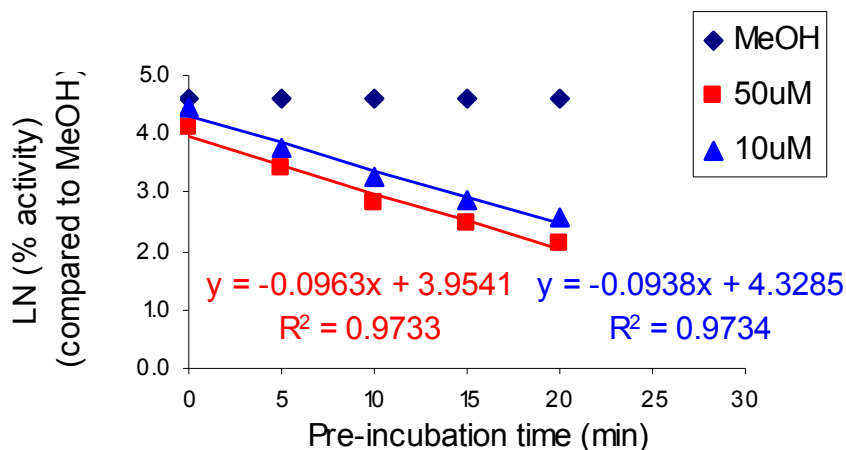
Mifepristone

→ Known to cause TDI

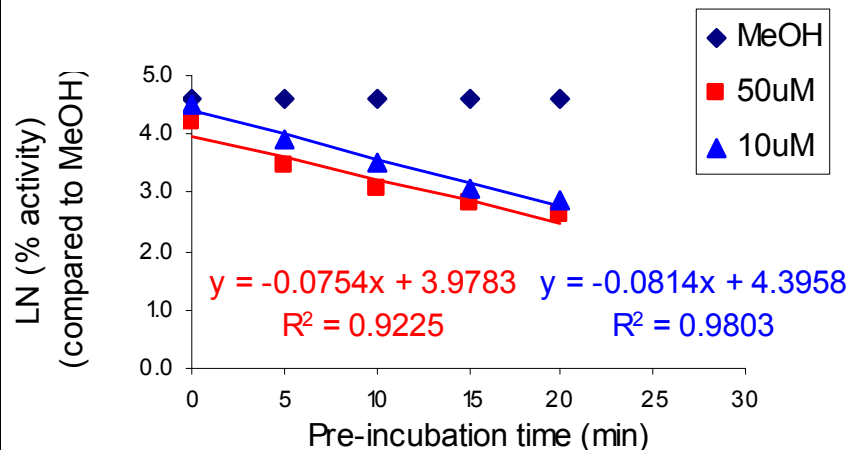
HPLC-MS/MS (API4000)

LDTD384-MS/MS (Quantum)

Time dependent Inhibition of Mifepristone



Time dependent Inhibition of Mifepristone



Comparable results by HPLC-MS/MS and LDTD384-MS/MS

Application #2 – Metabolic stability

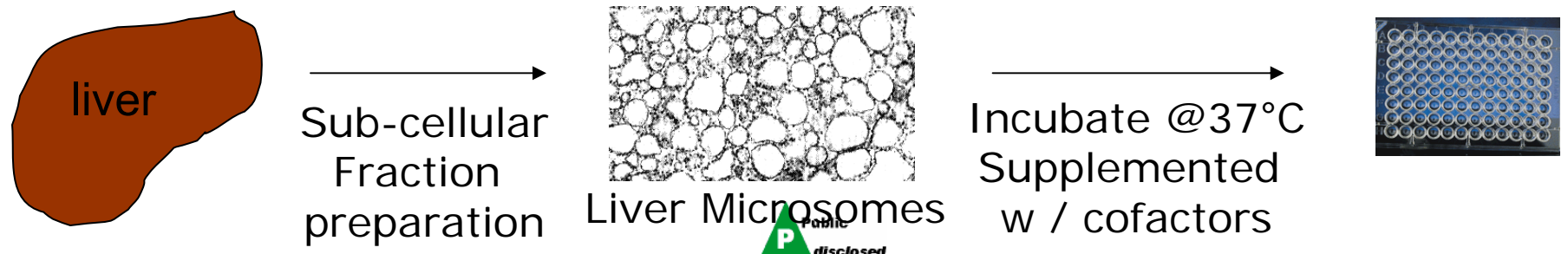
• Hepatocytes

- Cells involved in the detoxification, modification and excretion of exogenous substances

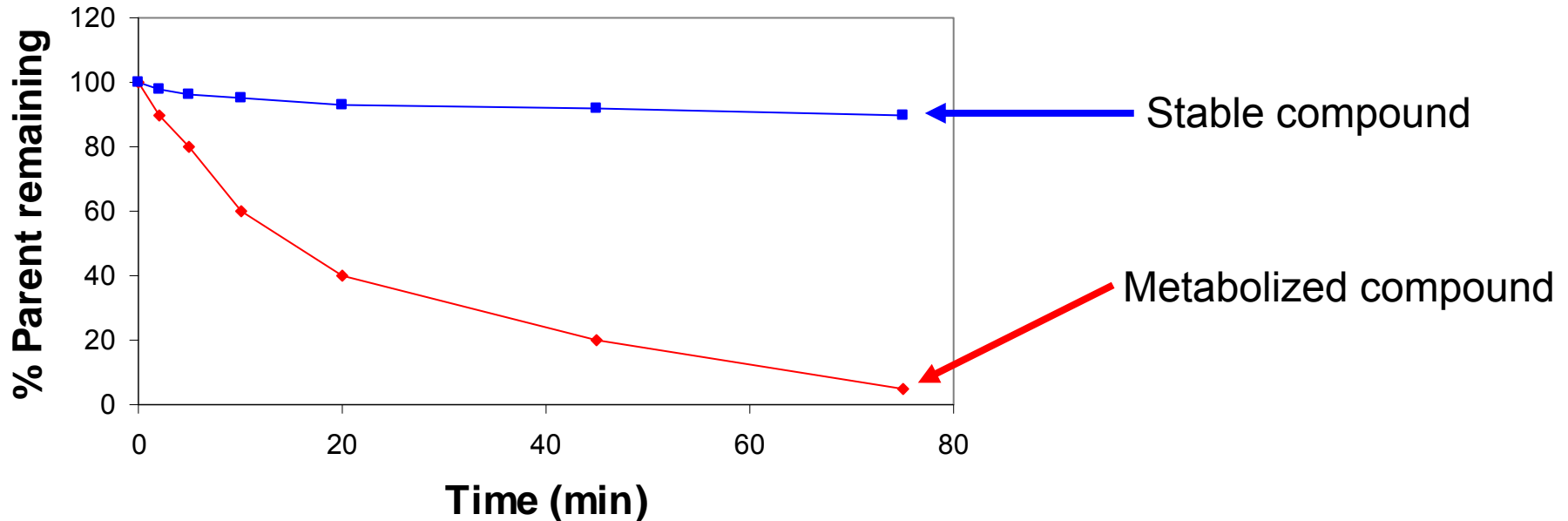


• Liver microsomes

- Concentrated Cytochrome P450 being a valuable tool for investigating the metabolism of compounds



Application #2 – Metabolic stability



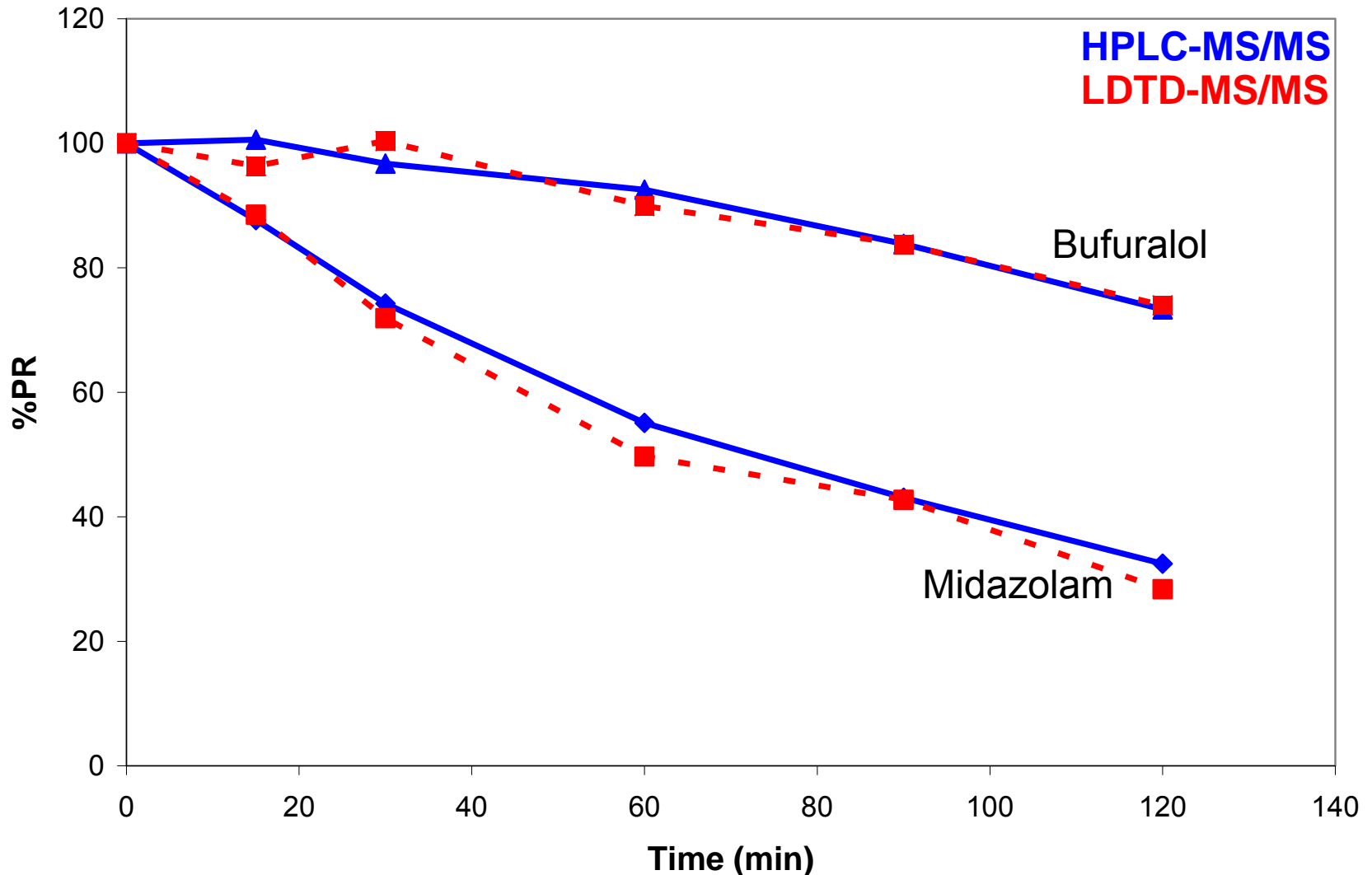
- MSIC in vitro assay

- Allow determination of half life (min), elimination rate (1/min) and in vitro Cl_{int} (mL/min/kg)

- Used to screen stable compound, PK prediction, etc.

Application #2 – Metabolic stability

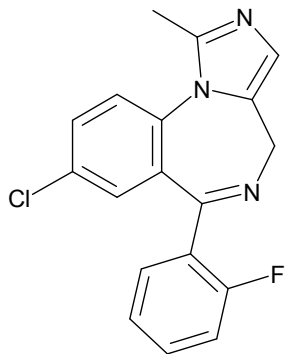
Human hepatocytes, 1e6 cells/mL, 37°C, 95/5% O₂/CO₂



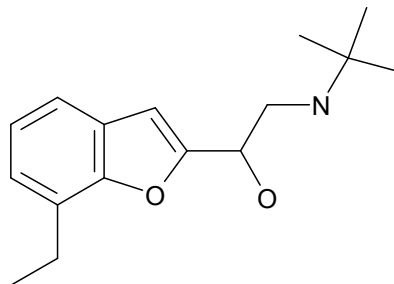
Comparable results by HPLC-MS/MS and LDTD384-MS/MS

Application #2 – Metabolic stability

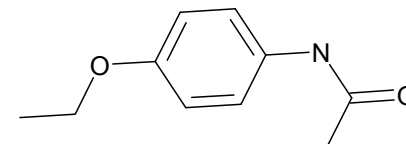
Liver microsomes, 0.25mg/mL, 37°C, with NADPH



Midazolam



Bufuralol

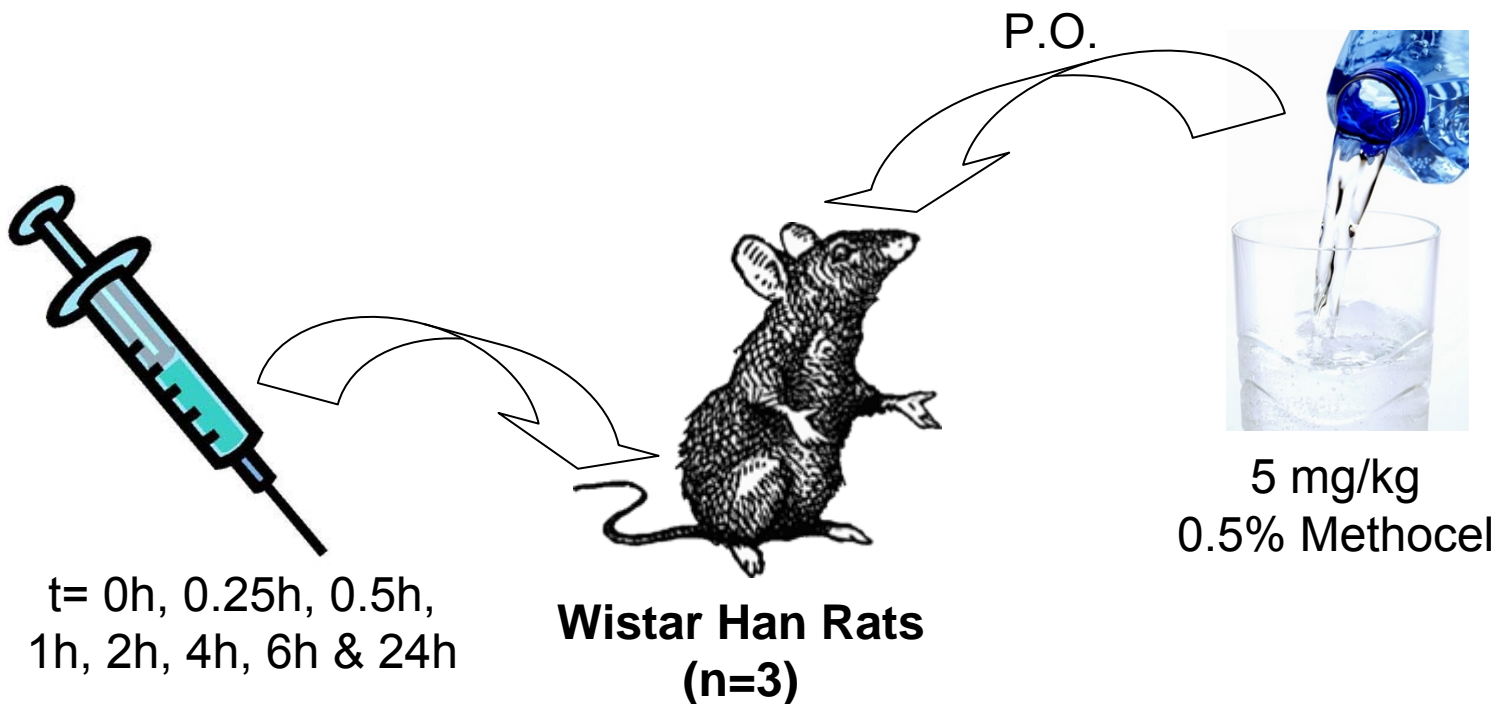


Phenacetin

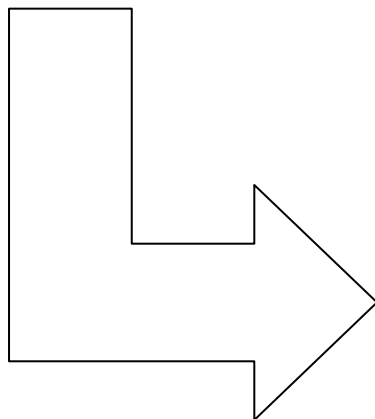
Compound	species	LDTD384-Quantum			HPLC-4000QTRAP		
		Half Life (min)	Elimination Rate (1/min)	In Vitro Clint (mL/min/kg)	Half Life (min)	Elimination Rate (1/min)	In Vitro Clint (mL/min/kg)
Midazolam	human	8.0	0.0865	288	4.2	0.1660	553
	dog	2.0	0.3443	1983	2.6	0.2673	1540
	rat	2.2	0.3220	>2000	1.7	0.4110	>2000
Bufuralol	human	79.4	0.0087	29	106.9	0.0065	22
	dog	26.0	0.0267	154	25.9	0.0267	154
	rat	3.7	0.1852	>2000	1.7	0.4084	>2000
Phenacetin	human	65.9	0.0105	35	80.2	0.0086	29
	dog	38.0	0.0182	105	24.2	0.0286	165
	rat	105.0	0.0066	48	65.4	0.0106	76

Data generated by LDTD384-MS/MS are within 2 fold of the data generated by HPLC-MS/MS

Application #3 – Pharmacokinetics



**Plasma quenched
with 3 volumes ACN**

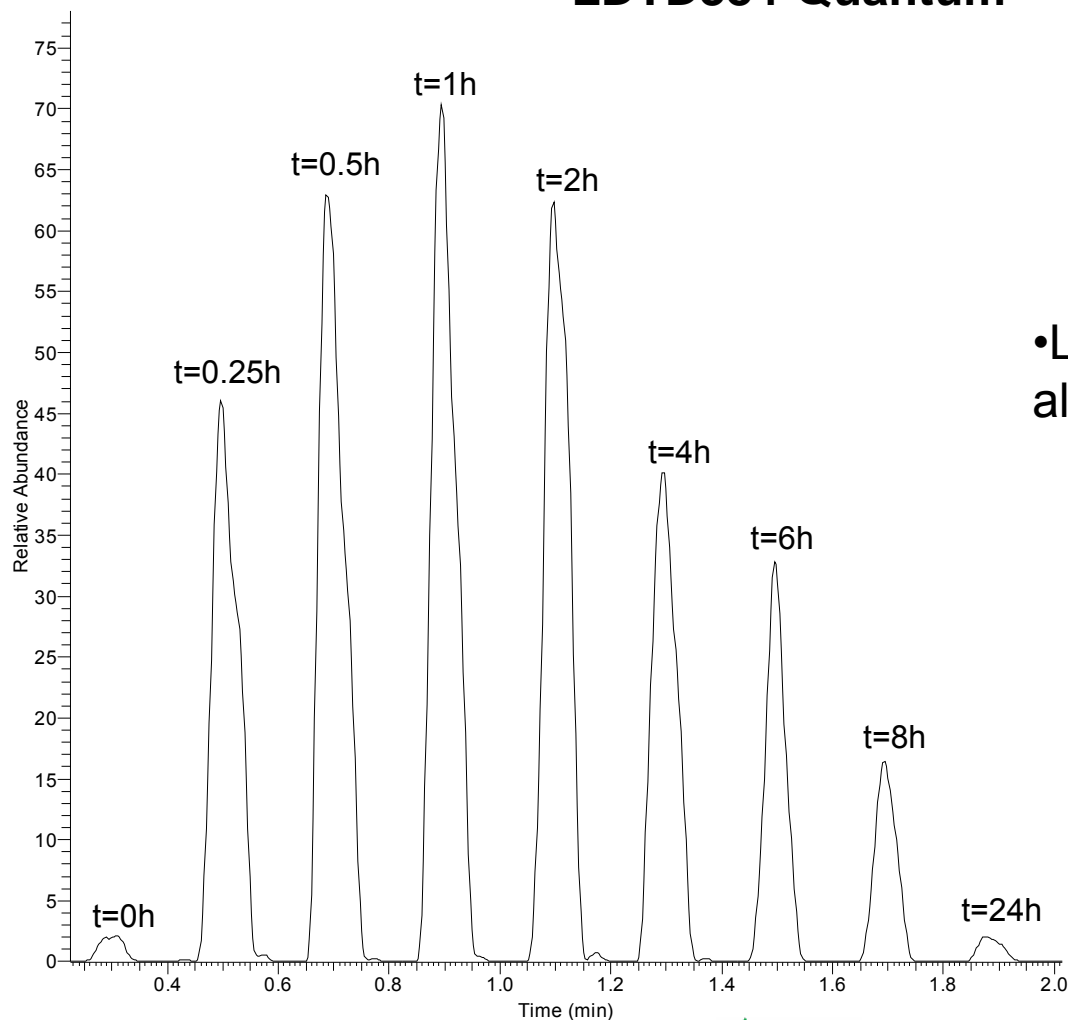


Application #3 – Pharmacokinetics

WH rat PO @ 5mg/kg in 0.5% Methocel

LDTD384-Quantum

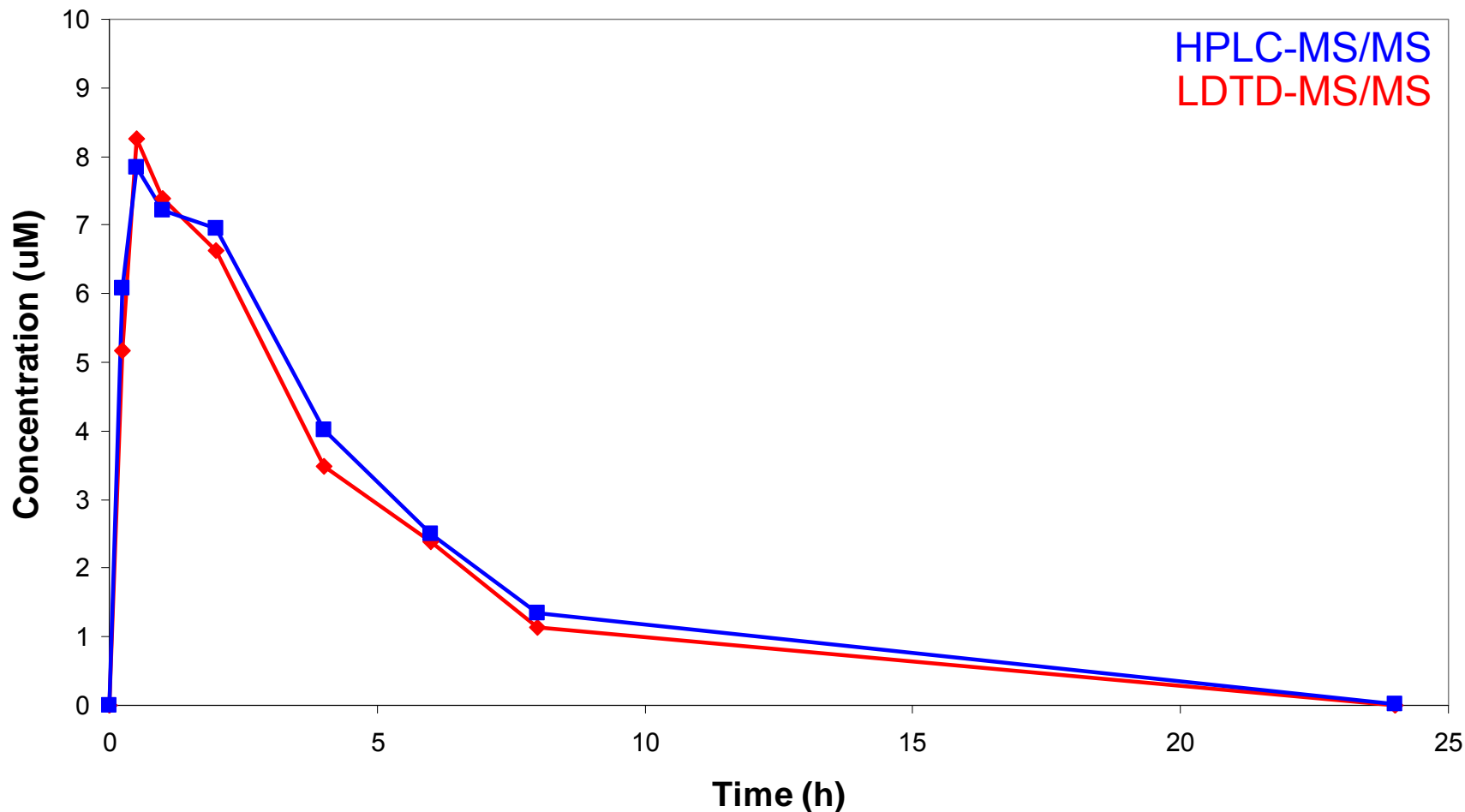
RT: 0.22 - 2.01 SM: 7B



•Less than 2 minutes to run
all plasma samples from 1 rat

Application #3 – Pharmacokinetics

WH rat dosed P.O. @ 5 mg/kg in 0.5% Methocel



Comparable results by HPLC-MS/MS and LDTD384-MS/MS

However, the LOD is 3 fold higher by LDTD384-MS/MS (1.5nM vs 5nM)

LDTD384-MS/MS: Pros & Cons

- **Pros**

- Fast → 12 seconds sample-to-sample
- Autonomy of 3840 samples (10x 384well plates)
- Small amount of sample needed (1uL)
- Compatible with all mass spectrometer

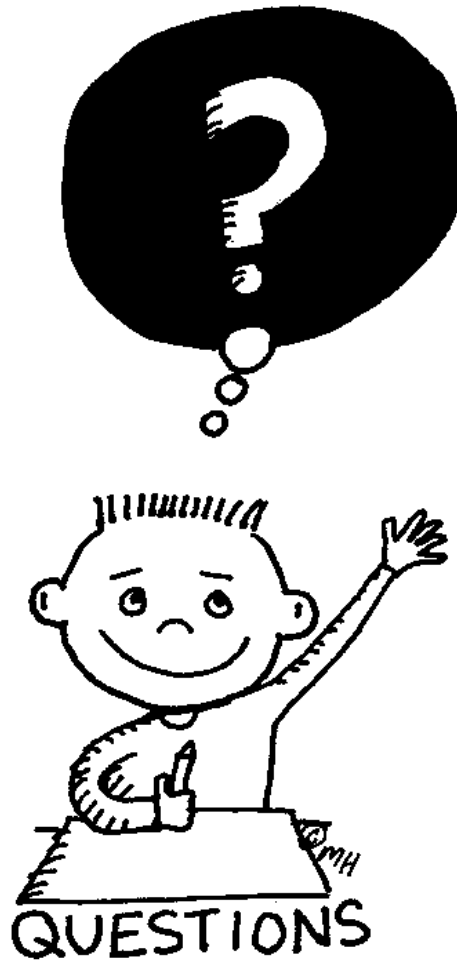
- **Cons**

- No separation
- Less compounds can be ionized vs ESI
- Some MRM with background
- LOD in plasma of 5nM (vs 1.5nM by HPLC-MS/MS)
 - Quantum vs API5000

Conclusion

- LDTD384-MS/MS is:
 - 25x faster per sample than conventional HPLC-MS/MS methods
 - requiring a change in the way that the samples are treated vs HPLC-MS/MS
 - A challenge in a discovery environment with different compounds daily
 - recommended for high throughput analysis of targeted compound (CYP inhibition for example)

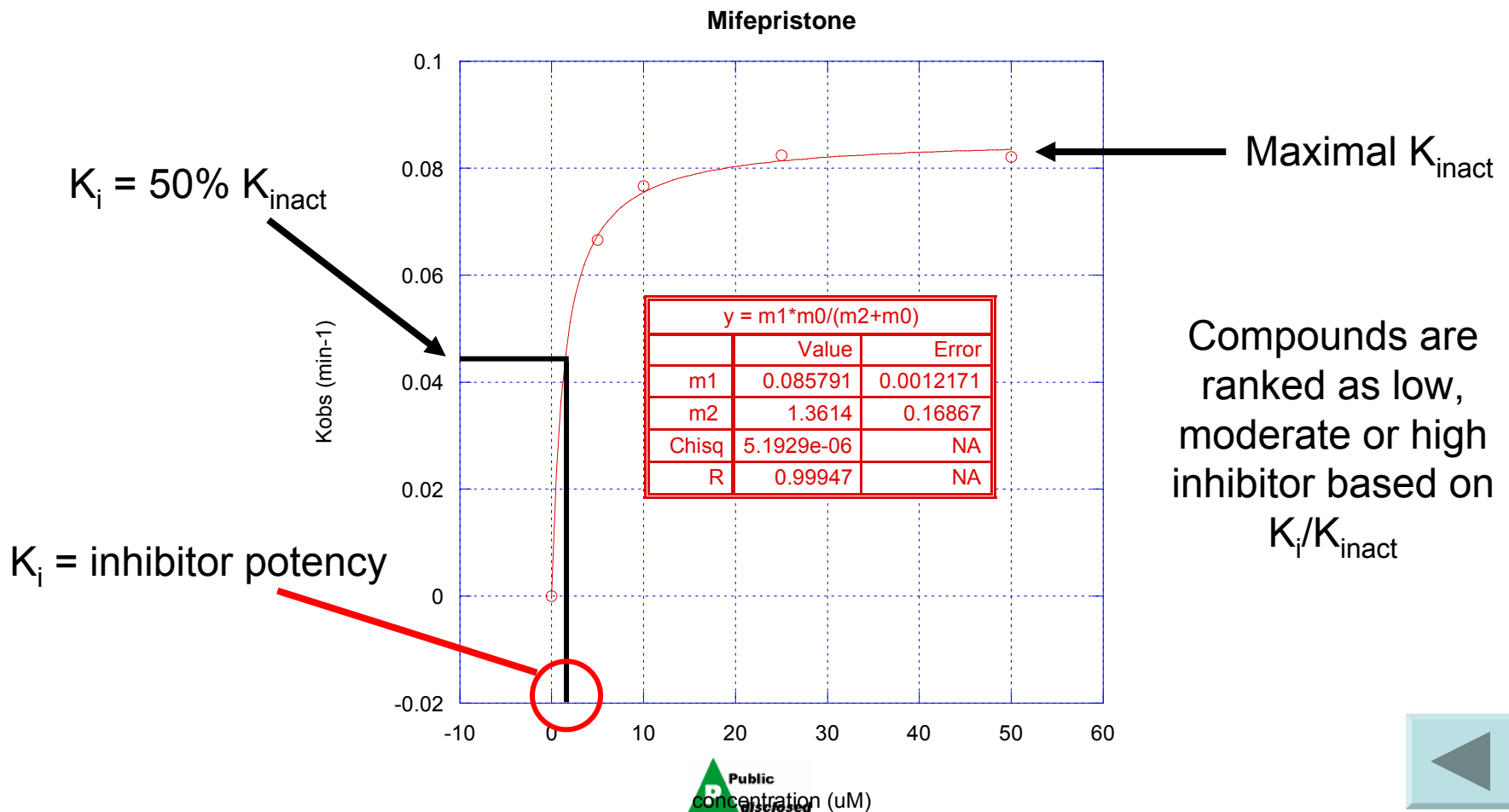
Questions



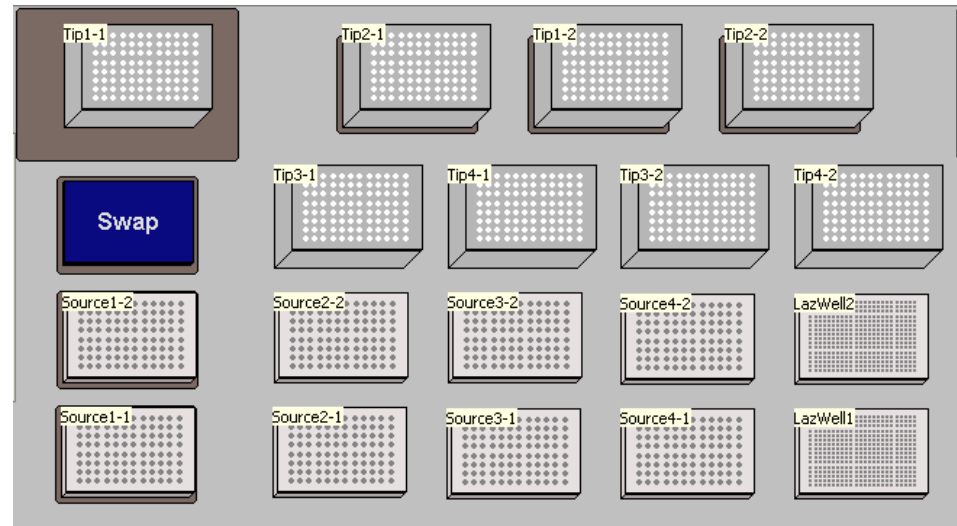
Back-up

K_i/K_{inact}

- K_i/K_{inact} → Test compound at 0, 5, 10, 25 & 50uM
- Pre-incubation: 0', 5', 10', 15', 20'

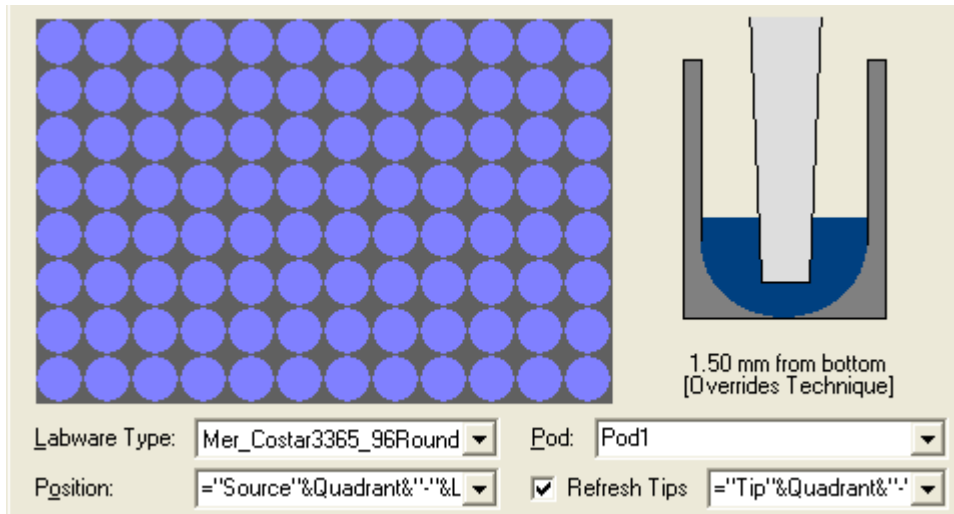


Biomek FX for optimal transfer to Lazwell plates

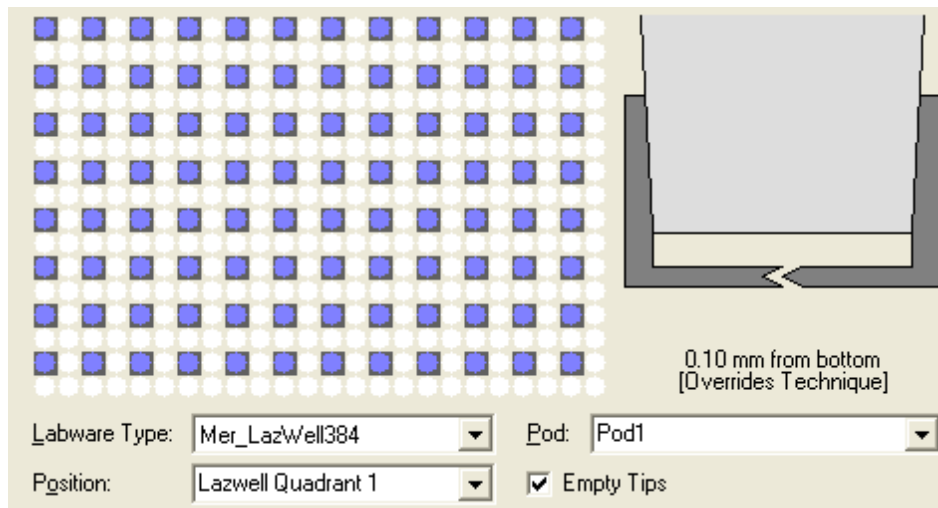


- Assay / samples preparation done in 96well format
- Pool 4x 96well plates on 1x 384Lazwell
- Transfer of 1uL are done using 10uL tips for optimal efficiency

Biomek FX for optimal transfer to Lazwell plates



- Sample pick-up
- 1uL accurately

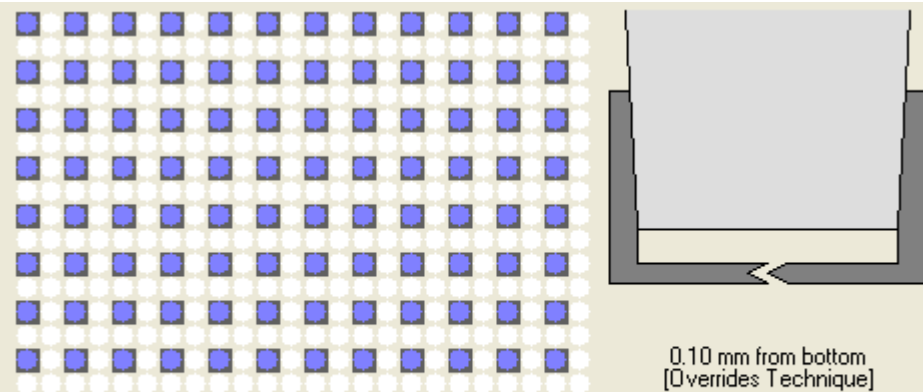


- Sample delivery
- 1uL accurately
- Sample can be delivered in selected Quadrant



Biomek FX for optimal transfer to Lazwell plates

QUADRANT 1

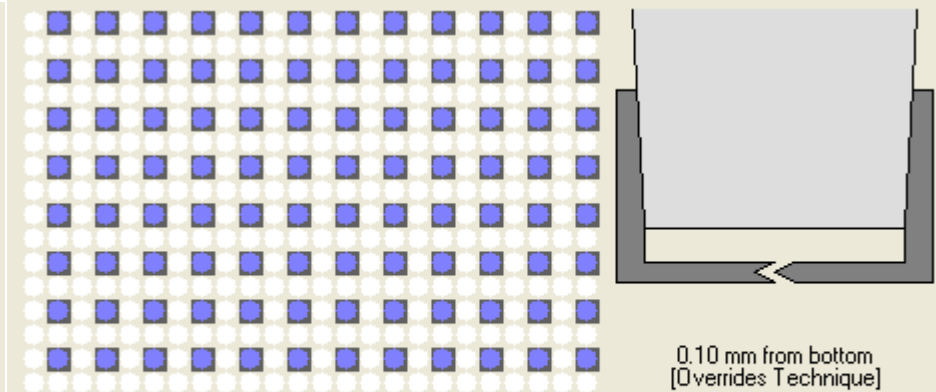


0.10 mm from bottom
[Overrides Technique]

Labware Type: Mer_LazWell384 Pod: Pod1

Position: Lazwell Quadrant 1 Empty Tips

QUADRANT 2

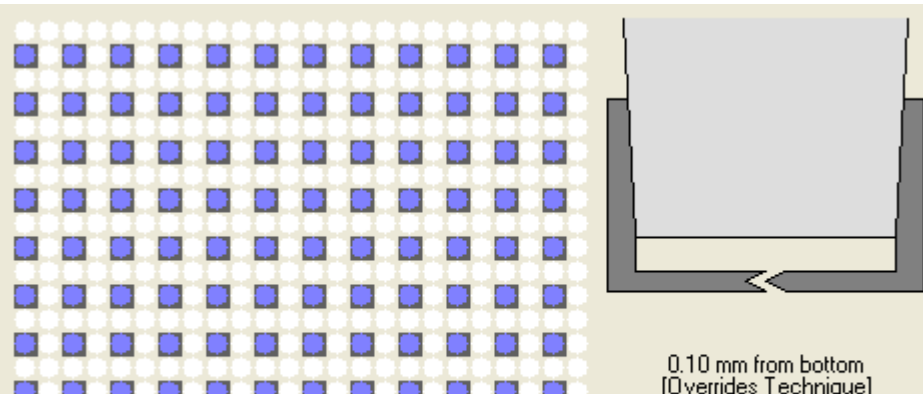


0.10 mm from bottom
[Overrides Technique]

Labware Type: Mer_LazWell384 Pod: Pod1

Position: Lazwell Quadrant 2 Empty Tips

QUADRANT 3

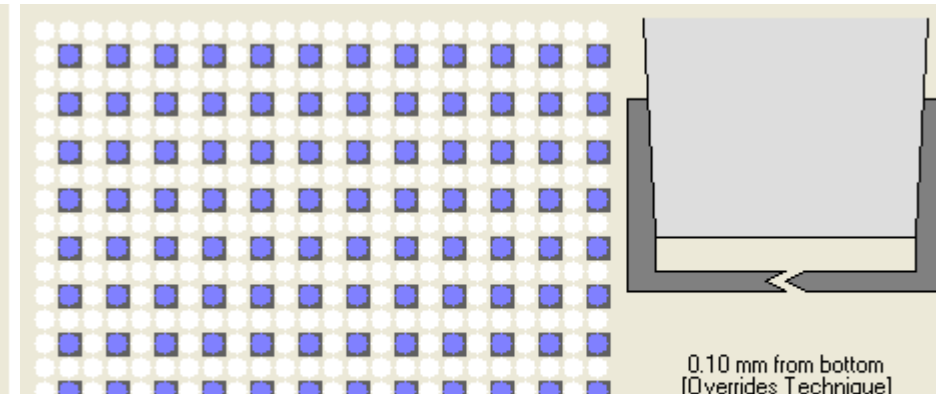


0.10 mm from bottom
[Overrides Technique]

Labware Type: Mer_LazWell384 Pod: Pod1

Position: Lazwell Quadrant 3 Empty Tips

QUADRANT 4



0.10 mm from bottom
[Overrides Technique]

Labware Type: Mer_LazWell384 Pod: Pod1

Position: Lazwell Quadrant 4 Empty Tips