

# LASER DIODE THERMAL DESORPTION (LDTD) MASS SPECTROMETRY FOR HIGH THROUGHPUT ANALYSES OF DISCOVERY DMPK IN VITRO AND IN VIVO SAMPLES.

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## Introduction

In Drug Discovery, particularly in DMPK, traditional High Pressure Liquid Chromatography (HPLC)-Mass Spectrometry (MS/MS) are prerequisite instruments used as the benchmark techniques for the determination of compound levels in various in vitro and in vivo samples. Although selectivity and sensitivity of the modern MS/MS allow for a simpler sample preparation and faster generic LC conditions via the use of modern Ultra High Pressure LC (UPLC or UHPLC), LC-MS/MS is still the limiting bottleneck step when working with hundreds of samples per assay per day. The objective of this study was to evaluate the new LDTD device, compare compound levels/results obtained from LDTD-MS/MS with conventional LC-MS/MS and assess its applicability in a Drug Discovery setting.

## Method

**LDTD** (Figure 1) is a thermal desorption technique induced by a laser diode that introduces samples from a LazWell (Figure 2) directly into the mass spectrometer without any chromatography steps

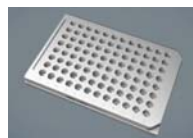


Figure 1: LDTD device mounted on the TSO Quantum Ultra ion max source (ThermoFisher)

Figure 2: 96 LazWell plate from Phytronix Technologies

In vitro and in vivo routine DMPK assay samples (samples from PK, CNS exposure, Metabolic Stability, Caco2 and Plasma Protein Binding experiments) were prepared into a conical polypropylene 96 well plate using simple protein precipitation techniques, Aqueous:Acetonitrile (1:2 to 1:10; v/v), vortex mixed and centrifuged at 9,000 x g at 4°C for 30 minutes.

Ten microliters of supernatant were injected using a generic LC-MS/MS method ESI mode. Two microliters of the same samples were deposited on a LazWell plate, air dried and Laser shot using LDTD-APCI-MS/MS. Generally, run times were 3 minutes for conventional LC-MS/MS technique and less than 10 seconds for the new LDTD-MS/MS method.

### Conventional LC-MS/MS methods

- Columns:
  - Hypersyl GOLD, 50 x 2.1 mm, 1.9µ
  - HyPurity C18 column, 50 x 2.1 mm, 3µ
  - Eclipse XDB-C18, 30 x 4.6 mm, 1.8µ
- Waters/Agilent binary LC system and a Waters 2777/Agilent 1100 autosampler
  - Mobile Phases: A=H<sub>2</sub>O (0.1% formic acid) B=ACN (0.1% formic acid)
  - Elution: 5%B - 95%B from 0-2 min, Flow: 0.75mL/min or 1mL/min
- Thermo TSO Quantum/ Waters Quattro Premier/Micro/Agilent MSD: ESI+ and SIM/MRM

### LDTD-MS/MS methods (Figure 3)

- Laser power pattern : 0-45% from 0.5-3.5 sec; hold 3 sec.
- APCI Corona voltage : 5 KV

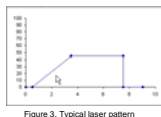


Figure 3: Typical laser pattern

## Statistical analysis

The presence of a significant bias or a significant lack of agreement between method were evaluated using the paired student t-test (p<0.05)

## Results

LDTD vs LC MS/MS comparison of In vivo rat intravenous and oral pharmacokinetics of selected AZRDM compounds (Figure 3 and Table 1)

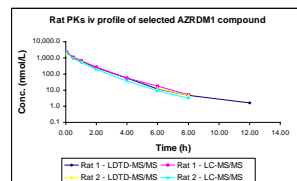
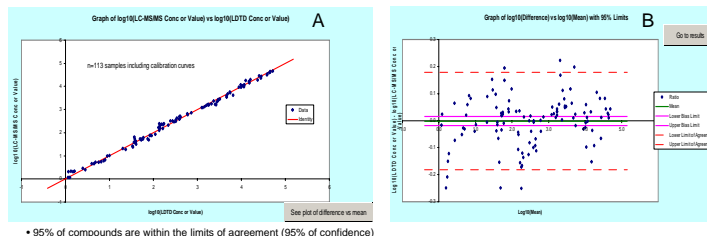


Figure 4: Typical Rat PKs iv profile of AZRDM1 proprietary compound

	AZRDM1		AZRDM2	
	LC	LDTD	LC	LDTD
i.v.				
AUC (nM)	2,012.9	1,982.6	2,389.4	2,277.3
AUC <sub>0-T</sub> (nM)	2,006.6	1,976.4	2,382.6	2,272.1
T1/2 (h)	1.04	1.04	1.02	0.94
CL (l/h/kg)	1.15	1.07	2.25	2.42
Vdss (l/kg)	1.03	1.12	2.11	2.69
p.o.				
Cmax (nM)	1,873.4	1,884.5	275.9	224.4
F(%)	>100	>94.7	23.52	17.95

Table 1: Rat PKs parameters of tested AZRDM1 and AZRDM2 proprietary compounds

## Figure 5: LDTD vs LC MS/MS relationship of in vivo mouse CNS penetration (Brain and Plasma) sample levels (A) and the difference vs its mean (B) of selected AZRDM compounds



95% of compounds are within the limits of agreement (95% of confidence)

## Figure 6. Typical intrinsic Clearance (CLint) of Dextromethorphan in human liver microsomes (HLM)

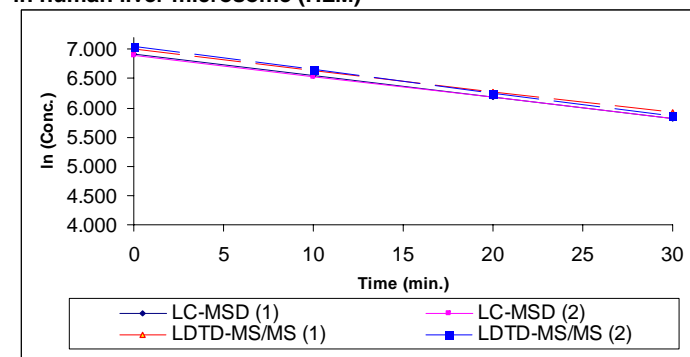


Table 2. HLM CLint of selected compounds obtained by LDTD-MS/MS vs LC-MSD

Compounds	HLM CLint (ul/min/mg)	
	LC-MSD	LDTD-MS/MS
Verapamil	171	187
	173	181
Dextromethorphan	75	85
	72	76
AZRDM3	43	20
AZRDM4	8	24
AZRDM5	16	23
AZRDM6	73	55

## Discussion and Conclusions

- Preliminary results obtained from the LDTD and LC-MS/MS show that:

- There is no significant difference observed between the relevant PK parameters
- There is no evidence of a statistical bias between the two analytical methods for CNS samples (p>0.05, n=113 samples)
- A good agreement between hCLint obtained values from both methods
- It is difficult to find an appropriate universal generic internal standard for the LDTD when working with diver classes of compounds in early drug discovery
- An increase of >20x in throughput (3 min. vs 9 sec.) with LDTD vs conventional LC-MS/MS
- One compound (e.g.: Warfarin) showing non reproducible results when analyzing with LDTD
- Plasma Protein Binding (PPB) samples showed some discrepancy and that could be due to slight different in matrix from the samples (equilibrium) vs calibration curves
- Limited volume of sample needed (2uL on LazWell plate)
- In summary, the LDTD can be a very powerful analysis tool in Discovery DMPK for high throughput screening of routine assays.

### Future perspectives:

- Increase the number of experiments to strengthen the finding results
- Evaluate samples containing exotic vehicles that might cause signal suppression or enhancement; e.g.: Cyclodextrin; PEG400; solutol...
- Evaluate the use of LDTD for Caco2
- Pooling samples from different experiments (different compounds)