

Application of a Laser Diode Thermal Desorption (LDTD) Ion Source for Mass Spectrometry in a Drug Discovery Environment

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Overview

- Goal**
 - To demonstrate a new laser diode thermal desorption (LDTD) ion source for rapid sample analysis with minimal sample preparation
- Methods**
 - LDTD: Laser-induced thermal desorption coupled with atmospheric pressure chemical ionization (APCI) on a triple quadrupole mass spectrometer
 - Sample: plasma samples from pharmacokinetic (PK) studies, incubations from cytochrome P450 (CYP) inhibition assays, and phospholipids
- Results**
 - Low nM sensitivity for many compounds
 - Dynamic range: 3-4 orders of magnitude; good reproducibility
 - LDTD/APCI-MS/MS works well for a wide range of *in vitro* vivo samples
 - One 96 well plate analyzed in <30 min (<18 samples)

Introduction

- The LDTD source**
 - Developed by Phytronix Technologies (Dartec, QC, Canada)
 - Indirect thermal desorption by a laser diode (880 nm, 20 W)
 - Atmospheric pressure chemical ionization (APCI)
 - Atmospheric pressure, fits with any mass spectrometer
 - Minimal sample prep, no LC separation
 - No solvent, no matrix application

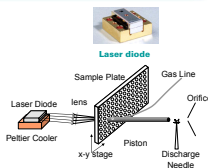
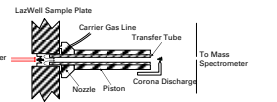


Figure 1. Schematic of the LDTD Source

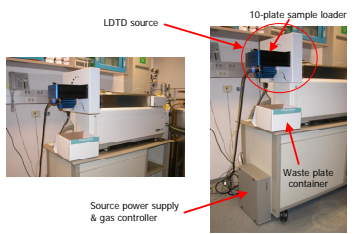


Methods

- Instrumentation**
 - LDTD source - Applied Biosystems/MDS SCIEX API 4000
 - LasWell sample plate
 - Standard 96-well plate format, disposable
 - Inserts/well bottom: proprietary stainless steel alloy
 - 1-10 µL of sample per well (no matrix)
 - No carrier: each sample has its own well
 - Amenable to robotic sample preparation systems
 - Current well design: hexagonal
- Experimental condition set up**
 - The SRM conditions of each analyte/internal standard were optimized by infusion of standard solutions into MS (+ESI)
 - 2 µL of each standard was spotted on the LasWell plate, dried, and laser power/duration and carrier gas conditions were optimized individually
 - Carrier gas for LDTD: compressed air, 50 °C, 2-2.5 L/min



Figure 2. LDTD Source on API-4000



PK and CYP Inhibition Samples

- Rat pharmacokinetic (PK) samples**
 - Three Sprague-Dawley rats were dosed (P.O.) at 10 mg/kg
 - Plasma samples were collected at 15, 30, 1 h, 2 h, 4 h, 5 h, and 6 h
 - Plasma samples were quenched with 2 volumes of CH₂Cl₂ followed by centrifugation to remove proteins
- CYP1A2/C2C8/D6/3A4 competitive inhibition assays**
 - Internal standards (IS): Stable isotope labeled compound of each metabolite
 - One assay per plate using different probe for each CYP isoform
 - Incubated in human liver microsomes HLM at 37 °C for 10 min for 3A4 & 2C8, 20 min for 2D6 & 1A2
 - Quenched each plate with equal volume of CH₂Cl₂ containing corresponding IS & centrifuged

Results and Discussion

General Characterization of LDTD-APCI-MS/MS

Figure 3. Optimization of LDTD Conditions: Laser Power & Carrier Gas Flow Rate

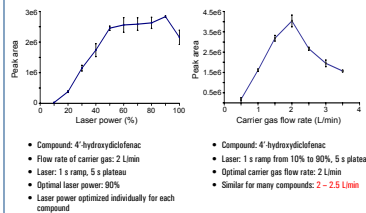


Figure 4. Sensitivity, Linearity and Reproducibility

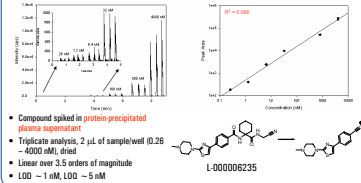


Table 1. Sensitivity, Linearity and Reproducibility

Compound	Dynamic Range	LOD	LOQ
4'-Hydroxydiclofenac	1.28 nM - 4 µM	1 nM	5 nM
Diclofenac	1.28 nM - 4 µM	1 nM	5 nM
Acetaminophen	4.4 nM - 4 µM	1 nM	5 nM

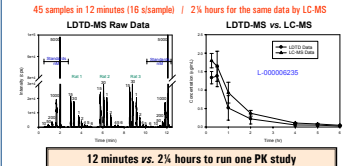
• Reproducibility measured for 6β-hydroxytestosterone (5 µM) in protein-precipitated HLM supernatant on a full 96-well plate
 • Peak area NCV ~ 10%
 • Peak area ratio NCV < 8% (with internal standard)

Applications of LDTD-APCI-MS/MS

Case 1: Pharmacokinetics (PK)

- Drug concentration vs. time profile
- Ideally would like minimum sample preparation and achieve fast turnaround in the drug discovery stage
 - protein precipitation (2 volumes CH₂Cl₂ volume plasma)
 - Pipet 2 µL of supernatant in each well
 - Analyze directly by LDTD-SRM
 - Low signal, without matrix optimization
- Compare with LC-MS analysis of the same samples
 - 10 µL injection
 - 10-90% CH₂Cl₂ on a 2x50 mm C18 column over 2 minutes
 - Total cycle time: 3 min/sample

Figure 5. PK Data: LDTD-MS vs. LC-MS



Case 2: CYP Inhibition Assays

CYP Isoform	Probe	Metabolite	MRM Transition	CE (eV)	Internal Standard
3A4	Testosterone	6β-Hydroxytestosterone	305.3→268.1	20	[16,14,17-D ₃]-6β-Hydroxytestosterone
2D6	Deutormethamphetamine	Dextrophan	258.3→201.0	43	[D ₃]-Deutrophan
2C9	Diclofenac	4'-Hydroxydiclofenac	312.2→231.1	28	[13C ₆]-4'-Hydroxydiclofenac
1A2	Phenacetyl	Acetaminophen	152.6→118.2	23	[13C ₆]-118 Acetaminophen

- LDTD Settings
 - 1 s ramp from 10-70% laser power, 5s plateau at 70% laser power
 - Carrier gas (compressed air) flow rate: 2.25 L/min

Table 2. Comparison of LDTD vs. ESI vs. APCI

Compound	ESI	APCI	LDTD	
6β-Hydroxytestosterone	Peak area (x 10 ³)	38 ± 3.7	120 ± 6.2	1800 ± 290
	SN	78	600	54000
Dextrophan	Peak area (x 10 ³)	2400 ± 170	3300 ± 300	1800 ± 42
	SN	11000	27000	6000
4'-Hydroxydiclofenac	Peak area (x 10 ³)	930 ± 40	2400 ± 190	6700 ± 260
	SN	107	4500	27000
Acetaminophen	Peak area (x 10 ³)	17 ± 3.9	7200 ± 240	12000 ± 1100
	SN	53	5400	12000

- 5 µM of compounds in 0.25 mg/mL HLM solution (protein-precipitated) (n = 3)
- LDTD: 2 µL samples, dried
- ESI & APCI: flow injection with a 5 µL loop @ 1 mL/min (50:50 H₂O/CH₃CN + 0.1% formic acid, no LC separation)

Figure 6. CYP2C9 Inhibition Assay

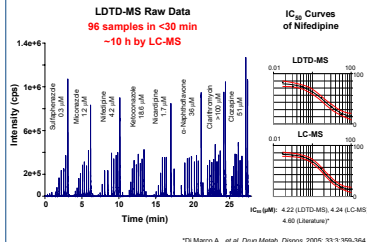
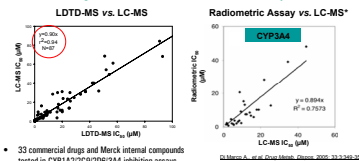


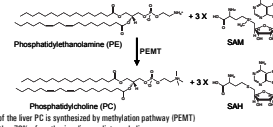
Figure 7. Correlation of CYP Inhibition Assays



- 33 commercial drugs and Merck internal compounds tested in CYP1A2/C2C8/D6/3A4 inhibition assays
- LDTD-MS data comparable to the standard LC-MS assay
- LDTD-MS data comparable to the literature high-throughput radiometric assay

Case 3: Phospholipid Analysis

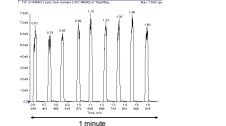
- Phosphatidylcholine (PC) synthesis in the liver via PE and a methyl donor



- 30% of the liver PC is synthesized by methylation pathway (PEMT)
- The other 70% of synthesis relies on dietary choline

Figure 8. Analysis of PC 18:1-C18:1

- 9 samples/min by LDTD-MS (~7 samples), ~1 sample/min by HPLC injection MS



Conclusions

- LDTD involves no matrix application, no LC separation, requires minimal sample preparation, consumes small quantity of samples, and has no carryover
- Sensitivity, linearity, reproducibility, and resistance to matrix effect make LDTD/APCI-MS/MS suitable for high throughput analysis in a discovery setting.
- High throughput analysis of PK, CYP inhibition and phospholipids have been demonstrated.
- LDTD/APCI-MS/MS can reduce analysis time from ~10 h to <30 min for a 96-well plate (can be as fast as <10 s/sample).
- Fully automated data acquisition for two 96-well plates. The 384-well plate version is under development by Phytronix.