Label-Free High Throughput Screening of Steroid using LDTD-MS/MS

Pierre Picard, Alex Birsan, Serge Auger and Jean Lacoursière, Phytronix Technologies, Québec, CANADA

OVERVIEW

Purpose

- Label-free high throughput screening of steroid from cell-based assays

Method

- Cell incubation with and without inhibition co-factor
- Assay incubation, quench and hydrolysis
- Extraction and deposition to analysis plate
- LDTD-MS/MS quantitative analysis in 1.9 seconds sample to sample
- Data analysis with MultiQuant™ software

INTRODUCTION

Screening drug candidates effect for potential activity is an important aspect in drug discovery. Label-free mass spectrometric measurement offers unmatched specificity and flexibility. This label-free screening using physiologically relevant substrates has increased the demand for mass spectrometry (MS) based analysis. Improving MS throughput, in a true high-throughput screening environment, remains a challenge for conventional ionization sources.

A recent publication[1] by Bristol-Meyers Squibb in the Journal of Biomolecular Screening (2015) demonstrates the advances made using the Laser Diode Thermal Desorption (LDTD) ion source interfaced with tandem MS as new high throughput solution enabling analysing time of < 1.9 seconds sample to sample (per well) for CYP analysis. The workflow was coupled with automated liquid handling system using samples of 1 µL deposited onto a 384-LazWell plate for LDTD.

Analysis of steroid based molecules in mass spectrometry may be challenging for liquid based ionization source. The LDTD ion source analyzes dried steroid samples with excellent sensitivity.

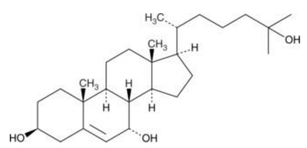


Figure 1 7-25-Dihydroxycholesterol

[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

LDTD® Ionization Source:

The LDTD® uses a Laser Diode to produce and control heat on the sample support **(Figure 2)** 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 1.9 seconds sample-to-sample analysis time, without carry over.

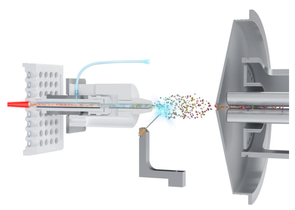


Figure 2 Schematic of the LDTD ionization source

METHOD

Sample preparation:

Reaction Component/Step	Vol /well; Time	Instrument
Test Samples reaction	4000 cells/well	Incubator
Ascorbic acid (prevent oxydation)	10 µL @ 5 mg/mL	Tecan Evo 150
IS solution	10 µL whole plate	Tecan Evo 150
Saponification solution	10 µL @ KOH (1.5N) + Na2SO3 (1.5N)	Tecan Evo 150
Vortex	3 minutes	TeShake module
Incubate	50°C, 1 hour	Incubator
Extraction	40 µL H2O and 200 µL Hexane/EtAc (9:1)	Tecan Evo 150
Process	Dispense 1 µL onto LazWell plate	Tecan Evo 150

Mass spectrometer settings:

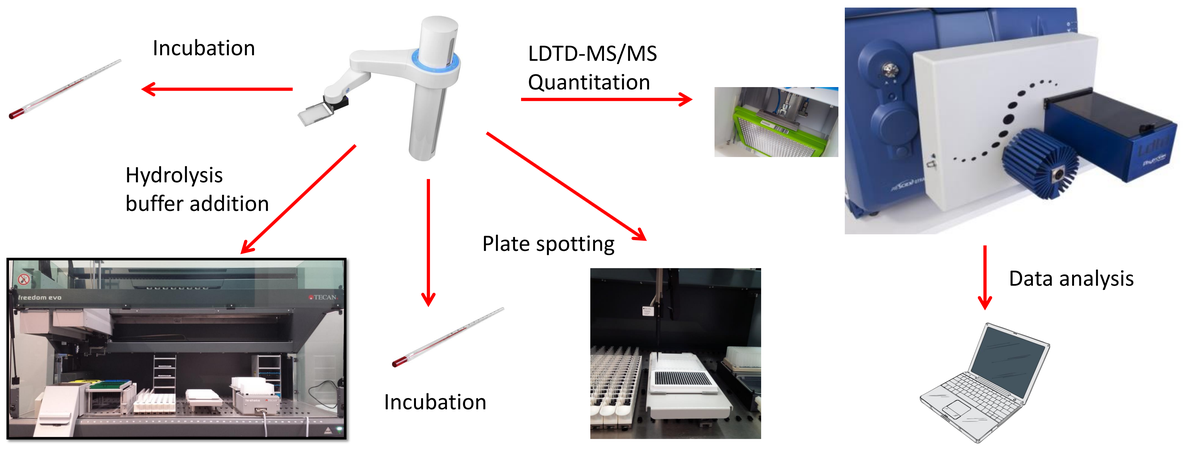
MS Parameters

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

LDTD Parameters

- Laser power pattern :
  - Increase laser power to 65 % in 1 sec
  - shut off
- Carrier gas flow (Air) : 3 L/min
- Model S-3840 with plate stacker

WORKFLOW



RESULTS

Analysis speed:

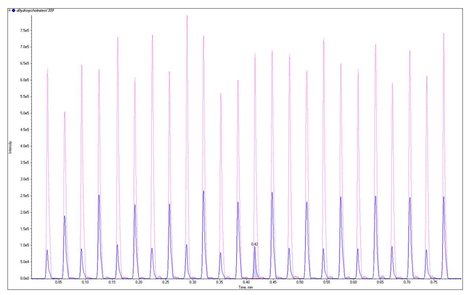


Figure 3 Desorption peak profile (zoom)

One row of 24 samples in 0.78 minutes:
1.9 sec/sample

- Alternating samples with/without inhibitor
- Optimized desorption time for signal intensity and reproducibility

Assay performance:

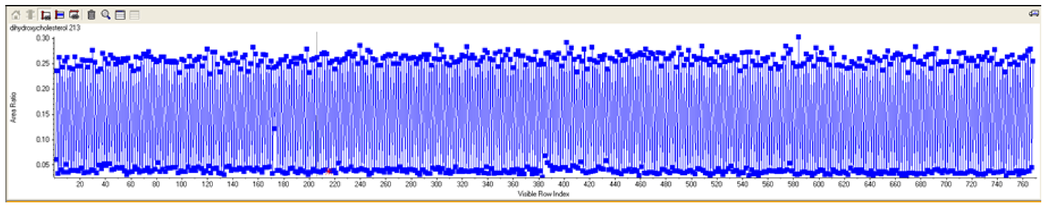


Figure 4 Desorption peak profile (all runs)

- 2 plates run consecutively for 768 samples
- Extraction from 8 96-well plates culture

	Low Level	High Level
Average Area ratio	0.0377	0.2585
SD	0.0053	0.0116
%CV	14.2	4.5

High Desorption Efficiency (HDE) plates:

To improve the desorption efficiency of steroids, high desorption efficiency plates are evaluated. These plates have a polymeric layer coating which changes the surface tension of liquids. The 1 µL of liquid is more focussed in the center of the well than for regular plates. This result in an increase of 22% in signal area count. Reproducibility is also improved.

	Regular Lazwell	Lazwell HDE
Average Internal standard Area count	0.0377	0.2585
Low level ratio reproducibility (%CV)	8.8%	6.0%
High level ratio reproducibility (%CV)	9.3%	3.3%

CONCLUSION

- **Label-free High Throughput screening of steroid in cell-based assay**
- **Accurate Area ratio for inhibition measurement**
- **Continuous operation with robotic arm and plate stacker**
- **Desorption improves by using HDE analysis plates, giving 22% more signal and better reproducibility**
- **Throughput of 1 sample every 1.9 seconds**
- **Possibility of assay miniaturization, signal allows the use of 500 cells instead of 4000**
- **Future work necessary since the actual culture cells plates are not suitable for quantity reduction as boundary effects are observed**