High Throughput LDTD-MS/MS Determination of Reserpine : 1000 Samples in less than 2 Hours

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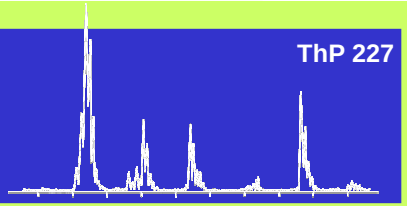


OVERVIEW

Purpose
– High-throughput determination of small molecules with sensitivity and repeatability
– Novel ion source based on Laser Diode Thermal Desorption technology (LDTD)

Method
– Reserpine spiked in human plasma
– Plasma precipitation using acetonitrile
– Calibration range : 0.5 ng/mL to 150 ng/mL
– Target sample concentration : 7.00 ng/mL
– LDTD-APCI-MS/MS analysis : Laser Diode Thermal Desorption coupled with triple quadrupole mass spectrometer

Results
• Excellent linearity over the calibration range ($R^2 > 0.99$)
• No matrix effect
• No carryover
• Excellent repeatability (10 %)
• Ultra-fast sample-to-sample analysis : **7.2 seconds per sample** **(2.5 seconds for sample desorption)**

INTRODUCTION

The high throughput determination of small molecules has become an important part for the pharmaceutical industries, such as drug discovery and quality control. It is also an economical issue for many contract research organizations (CRO). A novel ion source based on thermal desorption using a laser diode was used to perform high throughput analysis of reserpine, a well-known reference compound in mass spectrometry. The characteristics of the LDTD system are :

LazWell™ Plate (Figure 1)
– Standard 96-well plate format
– Low volume delivery (from 2 to 10 µL of sample per well)
– No carryover
– No enhancement matrix needed
– No liquid mobile phase needed
– Sample dried at room temperature

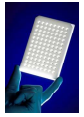


Figure 1 LazWell™ sample plate

LDTD (Figure 2)
– Plug-and-play ionization source interface to most popular mass spectrometer
– Thermal desorption induced by laser diode
– The sample is carried by a carrier gas to corona discharge region for APCI
– Loader up to 10 LazWell™ plates

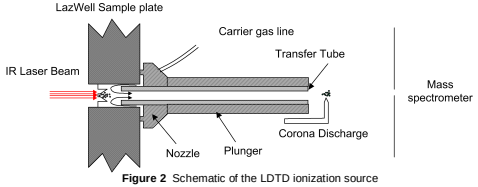


Figure 2 Schematic of the LDTD ionization source

METHOD

Instrumentation (Figure 3)
– LDTD model T-960, Phytronix Technologies
– Thermo Scientific TSQ® Quantum™ Ultra AM

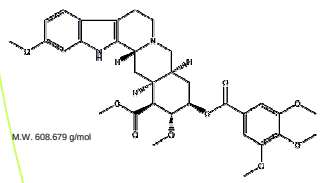
Figure 3 LDTD-MS/MS analytical system

LDTD Parameters
– Laser power pattern
  – Increase laser power to 25 % in 2.0 s
  – Hold at 25 % for 0.5 s
  – Decrease laser power to 0 % in 0.01 sec
– Carrier gas flow : 2.0 L/min (Air)
– Carrier gas temp. : 50 ºC
– Corona voltage value : 6 kV
– Deposited sample volume: 2 µL

MS Parameters
– MS/MS transition : 609.30 amu to 195.06 amu
– Collision gas pressure : 1.5 mTorr (Argon)
– Collision energy : 38
– Scan time : 0.02 s
– Q1 width : 1.00 amu
– Q3 width : 1.00 amu

Sample Preparation
– 100 µL human plasma with EDTA
– 500 µL of acetonitrile (precipitation agent)
– Vortex for 10 s
– Centrifuge at 14000 RPM for 10 min
– Filtrate supernatant on Nanosep 0.2 µm
– Spike reserpine in filtrated supernatant (7 ng/mL)
– **No internal standard added**
– Transfer **Manually** 4.0 µL onto LazWell™ to perform LDTD-MS/MS analysis
– **14 pg loaded in well**

Chemical Structure of Reserpine



RESULTS

Reserpine Calibration Curve

Quantitative determination of reserpine in human plasma can be achieved over a nominal concentration range from 0.5 to 150 ng/mL (**Figure 4**). An excellent linearity is obtained over the concentration range ($R^2 > 0.99$).

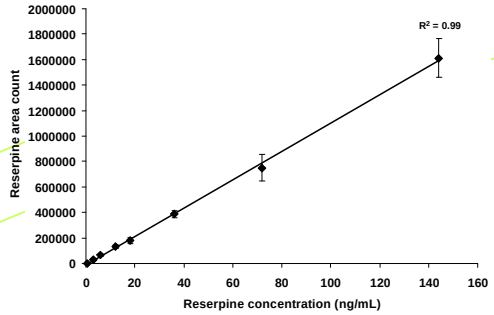


Figure 4 Reserpine calibration curve in spiked protein precipitated human plasma

Reserpine LDTD Profile

The Laser Diode Thermal Desorption process performed in **2.5 seconds** allows excellent Reserpine signal at a nominal concentration of 7 ng/mL (14 pg loaded in well). A complete LazWell™ plate (96 samples) analysis is performed in less than 12 minutes (**Figure 5A**). No carryover, no overlapping peak and no matrix effet is observed (**Figure 5B**).

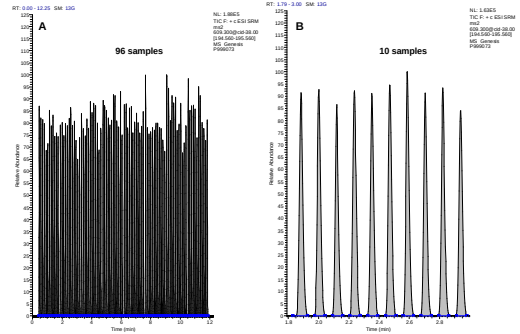


Figure 5 Reserpine sample analysis A) 96 samples corresponding to 1 sample plate, and B) 10 consecutive samples.

Reserpine Analysis Repeatability

Analysis of 960 reserpine samples at 7 ng/mL (14 pg loaded manually in sample plate) without internal standard correction gives a repeatability of 10.2 % (based on CV evaluation). As shown in **Figure 6**, the signal is constant all over the run, showing the stability of the LDTD-APCI process involved.

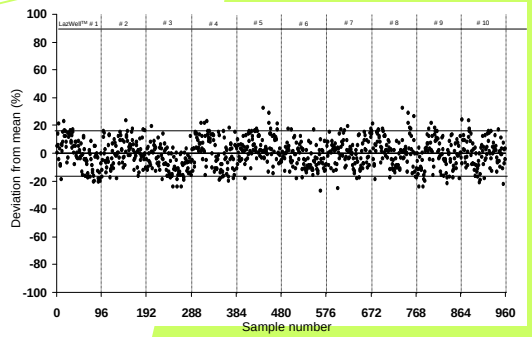


Figure 6 960 reserpine samples analyzed with LDTD-MS/MS

High Throughput LDTD Analysis

The reserpine analysis sequence :
- Coronna discharge stabilization (2 seconds)
- Sample desorption (2.5 seconds)
- Baseline stabilization (1.5 seconds)
- Well-to-well displacement (1.2 seconds)

These steps lead to a sample-to-sample run time of 7.2 seconds offering high troughput reserpine analysis.

CONCLUSIONS

– **Ultra-fast reserpine thermal desorption in 2.5 seconds**
– **Good signal repeatability through 960 samples analysis**
– **Excellent reserpine signal linearity in relation with concentration**
– **No matrix effect**
– **No carryover**
– **High Throughput analysis : 960 samples analyzed in 1.9 hours**