

High Throughput Analysis of 960 Reserpine Samples in 1.9 Hours

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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.

Goals

- Illustrate the efficiency of the LDTD-APCI source for reserpine analysis in 7.5 s per sample;
- Evaluate the working calibration range, the robustness and the repeatability of the LDTD-MS/MS in high throughput situation.

Instrumentation

- Phytronix Technologies LDTD ionization source (model T-960);
- Thermo Scientific Corporation TSQ[®] Quantum[™] Ultra AM mass spectrometer.

LDTD ionization process

The LDTD source uses an infrared laser diode to desorb samples that have been dried onto a well of the LazWell[™] (96-well plate). The desorbed gas phase molecules are carried over by a carrier gas into a corona discharge region to undergo APCI and then transferred directly into the mass spectrometer.

Samples Preparation

Protein precipitated human plasma (with plasma:acetonitrile 1:5) was filtered on Nanosep 0.2 µm. Reserpine was spiked directly in the treated human plasma. No internal standard was added to the solution. A volume of 4.0 µL of the sample was manually transferred into the well of a 96-well plate and was allowed to dry at room temperature.

LDTD Parameters

Discharge stabilization time	2.0 s
Laser power pattern	0 to 45 % in 2.0 s Hold at 45 % for 0.5 s Decrease to 0 % in 0.01 s
Baseline stabilization	1.5 s
Well-to-well displacement	1.2 s
Carrier gas flow	1.9 mL/min (Air)
Carrier gas temperature	50 °C

MS Parameters

Collision gas pressure	1.5 mTorr (Argon)
Collision energy	38 V
Scan time	0.02 s
SIM width	0.7 amu
Needle voltage	4500 V
Q1 and Q3 width	1.00 amu
Precursor mass	609.3 amu
Product mass	195.1 amu

Results and Discussion

Reserpine calibration curve

Quantitative determination of reserpine in human plasma can be achieved over a nominal concentration range of 0.5 to 150 ng/mL (**Figure 1**). An excellent linearity is obtained over the concentration range ($R^2 > 0.99$).

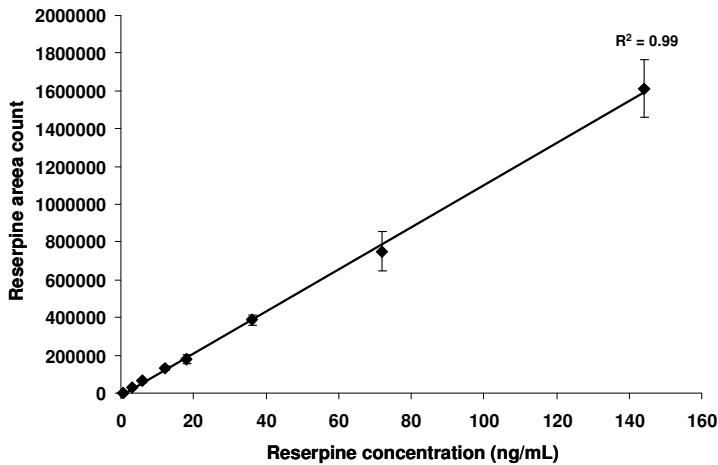


Figure 1 Reserpine calibration curve in spiked protein precipitated human plasma

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 2A**). No carryover, no overlapping peak and no matrix effect is observed (**Figure 2B**).

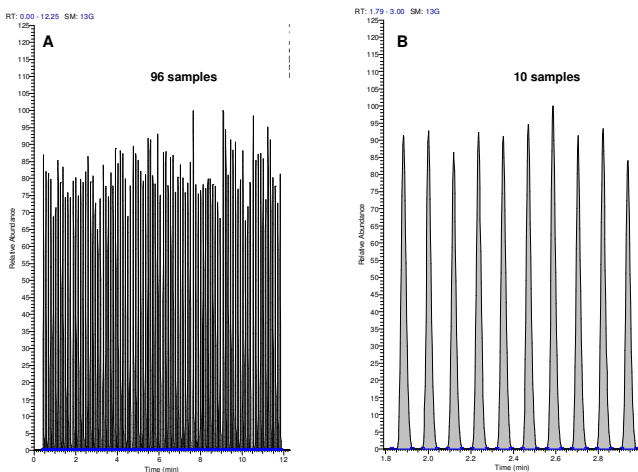


Figure 2 Reserpine sample analysis A) 96 samples corresponding to 1 sample plate, and B) 10 consecutive samples.

standard correction gives a repeatability of 10.2 % (based on CV evaluation). As shown in **Figure 3** the signal is constant all over the run, showing the stability of the LDTD-APCI process involved.

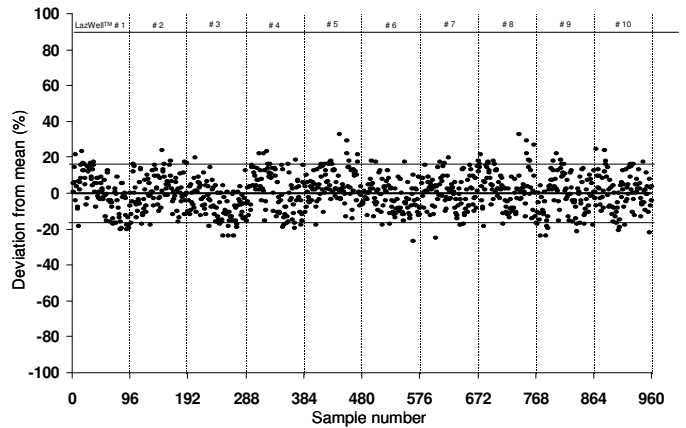


Figure 3 960 reserpine samples analyzed with LDTD-MS/MS performed in 1.9 hours.

Conclusions

LDTD-MS/MS allows ultra-fast reserpine thermal desorption in 2.5 s with an sample-to-sample run time of 7.5 s including the corona discharge stabilization. The produced LDTD-MS/MS signal is stable over 960 samples with no matrix effect and no observed carryover at 7ng/mL.

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