WP 153

Enabling Nanoliter sample size in Mass Spectrometry analysis using Laser Diode Thermal Desorption: application to pharmaceutical matrices

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OVERVIEW

Purpose
- Evaluate capability of nanoliter sample size analysis in LDTD-MS/MS

Method
- Plasma protein precipitation and CYP culture media organic quench

Quantification
- Excellent linearity (r=0.9996 to 0.9998) for all volumes spotted 0.1, 0.2 and 2 µL
- Conservation of effective quantitation within 2 folds decrease despite signal intensity reduction of 20x
- **Samples of 100 nL analyzed in 6 seconds using LDTD-MS/MS system**

INTRODUCTION

Do more with less is the pharmaceutical laboratories operational goal. Reduction of sample volume used for screening and study analysis represents a major impact on research and development cost. Based on this sample reduction approach, a study of low volume sample extract analysis in the nanoliter range is performed. Improvements in mass spectrometer sensitivity enable the possibility of using nano-sample size. Two different matrices are evaluated as typical work platform: Precipitated Rat plasma and CYP inhibition buffer. LDTD-MS/MS offers the possibility of using very low volume samples for high-throughput applications.

LDTD® Ionization Source:

The LDTD uses a Laser Diode to produce and control heat on the sample support **(Figure 1)** which is a 96 wells 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 6 seconds sample-to-sample analysis time, without carry over.

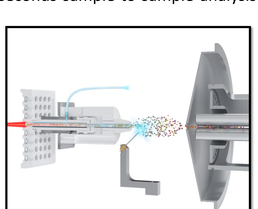

Figure 1 Schematic of the LDTD ionization source

METHOD

CYP inhibition Matrix:

- 50 µL buffered matrix spiked with Dextrorphan, OH-Testosterone and OH-Midazolam
- 450 µL of MeCN containing IS
- Vortex
- Deposit 0.1, 0.2 and 2 µL on plate
- Dry completely

Human Plasma:

- 50 µL of human plasma spiked with Clozapine
- 200 µL MeCN containing Clozapine D8
- Vortex
- Centrifuge
- Transfer 0.1, 0.2 and 2 µL of organic upper layer in LazWell™ plate
- Dry completely

Minimal sample size:

- 0.2 µL of human plasma spike with Clozapine
- 0.8 µL MeCN containing Clozapine D8
- Vortex
- Transfer 0.2 µL in LazWell™ plate
- Dry completely

Instrumentation
- LDTD model: S-960
- MS: Sciex 5500 QTrap®

LDTD Parameters
- Laser power pattern :
  - ➢ Increase laser power to 65 % in 3.0 sec
  - ➢ Maintain for 1 sec
  - ➢ Decrease laser power to 0 %
- Carrier gas flow (Air) : 3 L/min

MS Parameters
- APCI (+)
- Dwell: 50 msec
- Corona discharge: 3 µA
- DP: 100 V
- MRM mode (see **Table 1**)

Table 1 MRM method transitions

Compound	Q1	Q3	CE (V)
Clozapine	327.1	270.1	45
Clozapine d8	335.2	275.1	45
Dextrorphan	258.1	157.0	50
Dextrorphan d3	261.1	157.0	50
OH-Testosterone	305.1	269.0	20
OH-Testosterone d3	308.1	272.0	20
OH-Midazolam	342.0	203.0	27
OH-Midazolam d3	345.0	206.0	27

Figure 2 Micro sample size of 0.1 µL (color enhanced for photo)

Figure 3 Plasma Protein precipitation of 1 µL with 4 µL MeCN in eppendorf tube of 0.2 mL (color enhanced for photo)

RESULT

Analytical Quantitation:
- Limit of quantitation is determined by the effective signal to noise of the measurement
- In Thermal desorption, noise level varies during the heating step as shown in **Figure 4**
- LOQ are defined as the minimum signal which can be distinguish from background during heating period

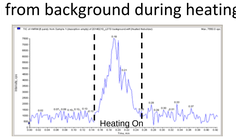

Figure 4 Background signal variation during thermal desorption

Effect of Small volume on Quantitation:
- State of the art mass spectrometer sensitivity enable use of small sample volume
- Expectation is to conserve LOQ based on signal to noise while intensity drop

Human Plasma Extract:
- Linearity remain excellent for 0.1 µL (**Figure 5**) with r^2=0.996 compared to 2 µL r^2=0.999
- For 20x volume reduction, signal is reduced by 10x and LOQ by 5X upon signal to noise level

Figure 5 Clozapine calibration curve of plasma protein precipitation, 0.1 µL deposited onto plate

CYP inhibition Matrix:
- Calibration curves ranging from 5 to 500 nM show excellent linearity for Dextrorphan, OH-Midazolam and OH-Mephenytoin for 0.1 µL and 2 µL
- Signal to noise level remain higher than 5 at LOQ for 0.1 µL deposited on plate
- Linearity remain equivalent for both sample size (**Figure 6**)

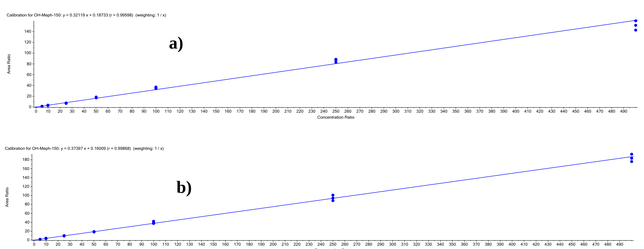

Figure 6 Calibration curves for OH-Mephenytoin a) 0.1 µL and b) 2 µL

CYP inhibition Matrix Multiplexing:
- Sample volume reduction allows the possibility of multiplexing extract with successive sample depositions in the same well without overloading
- All three multiplexed compounds show area counts within 15% of the individual value
- Good accuracy (105.8 to 107.5 %) and precision (1.2 to 3.6 %) are obtained at LLOQ level

CONCLUSION

- **Sample size reduction of 20x only reduces the attainable LOQ by a factor of 5X with LDTD-MS/MS**
- **Good precision and accuracy are obtained through different matrices and compounds. No carryover was observed.**
- **Ability of quantitate with accuracy from minimal sample was demonstrated.**
- **Small sample size is attainable and allows reduction of material consumed for large assays**