Dipyridamole high throughput analysis of drug metabolism cell-based assays using LDTD-MS/MS

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OVERVIEW

Purpose

- High throughput analysis of Dipyridamole in cell-based assays using LDTD-MS/MS

Method

- Protein precipitation cell-based assays to stop the reaction.
- Internal standard and desorption solution addition.
- LazWell plate spotting, evaporated to dryness and LDTD-MS/MS analysis

Quantification

- Linearity: $r^2 > 0.995$ over the calibration range
- Inter-run and Intra-run Accuracy ranging from 92.8 to 103.5%
- Inter-run and Intra-run Precision ranging from 3.7 to 9.7%
- **Samples analyzed with a run time of 5 seconds using LDTD-MS/MS system**

INTRODUCTION

Pharmaceutical laboratories aim to improve the throughput of the analytical chain. Reduction of analysis time for metabolism assay represents a major impact on research and development costs. In order to decrease the analysis time a high throughput method approach was developed and validated using a LUXON Ion Source, based on Laser Diode Thermal Desorption (LDTD), combined with an API 5500 Qtrap Mass Spectrometer. This high throughput analytical system allows measurement of Dipyridamole drug metabolite at a rate of 5 seconds per sample.

LUXON Ionization Source:

The LUXON Ion Source (**Figure 1**) is the second generation sample introduction and ionization source based on the LDTD® technology for mass spectrometry. LUXON Ion Source uses Fiber-Coupled Laser Diode (**Figure 2**) to obtain unmatchable thermal uniformity giving more precision, accuracy and speed. The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into 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 thermal desorption process yields high intensity molecular ion signal in less than 1 second sample to sample and allows working with very small volumes.

Figure 1 LUXON Ion Source

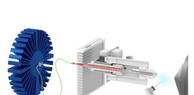

Figure 2 Schematic of the LUXON ionization source

METHOD

Sample preparation:

- A calibration curve of 0.5 to 200 ng/mL is prepared in a protein precipitated mixture
- Low, Medium and High QC levels are prepared in same mixture at 1.5, 30 and 160 ng/mL, respectively
- 100 µL of standard, QCs or test samples were mixed with 5 µL Internal standard solution.
- 10 µL desorption solution (BSA, 2 mg/mL) is added
- After mixture, 2 µL are deposited on LazWell plate
- LDTD-MSMS analysis after complete evaporation

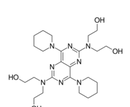

Figure 3 Dipyridamole structure

Figure 4 LUXON system on Sciex 5500 QTrap®

Instrumentation

- LDTD: LUXON
- MS: Sciex 5500 QTrap®

LDTD Parameters

- Laser power pattern :
  - ➢Increase laser power to 70 % in 3 sec
  - ➢Maintain power 2 seconds
  - ➢Decrease laser power to 0 %
- Carrier gas flow : 3 L/min (Air)

MS Parameters

- APCI (+)
- DP: 100 V
- Dwell: 50 msec
- MRM mode **(Table 1)**

Table 1 MRM transitions

Compound	Q1	Q3	CE
Dipyridamole	505.3	429.4	60
Dipyridamole-d_{20}	525.3	449.4	60

RESULTS

Linearity results:

A standard calibration curve ranging from 0.5 to 200 ng/mL has been prepared in a cell protein precipitated solution and analyzed. All curves have 0.995 coefficients or better. **Figure 5** presents a typical calibration curve for Dipyridamole.

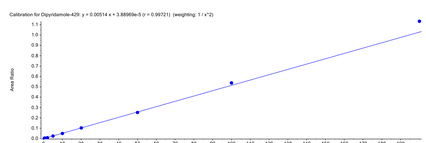

Figure 5 Typical Dipyridamole calibration curve

Intra-run

ULOQ, LLOQ, High, medium and low concentration QCs are added in the analysis. The intra-run accuracy and precision across the calibration curves are reported in **Table 2**.

Table 2 Dipyridamole intra-run results

	LLOQ	QC-Low	QC-Med	QC-High	ULOQ
Conc. (ng/mL)	0.5	1.5	30	160	200
N	6	6	6	6	6
Mean (ng/mL)	0.49	1.44	27.84	161.11	207.07
%RSD	9.7	8.4	7.7	4.8	3.7
%Nom	98.8	95.8	92.8	100.7	103.5

Inter-run

High, medium and low concentration QCs are added in the analysis. The inter-run accuracy and precision across the calibration curves were reported in **Table 3**.

Table 3 Dipyridamole inter-run results

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	1.5	30	160
N	18	18	18
Mean (ng/mL)	1.52	28.60	162.14
%RSD	7.0	5.9	4.4
%Nom	101.2	95.3	101.3

Carry over

Carry over is evaluated by analysis of three blank samples after high level calibrator. Peak area detected in blank sample is divided by the mean peak area value of LLOQ sample. Percentages of blank at LLOQ level are reported in **Table 4**.

Table 4 Carry over results

	% Blk interference	
	Analyte	Internal standard
BLK 1	2.4	0.03
BLK 2	0.2	0.01
BLK 3	0.1	0.01

Matrix interference evaluation

Six different matrices are evaluated for their interference at the Dipyridamole transition and internal standard. Peak areas detected in matrix test sample are divided by the mean peak area value of the LLOQ sample for the interference with Dipyridamole and the same evaluation is done for the internal standard. Percentages of matrix interference are reported in **Table 5**.

Table 5 Matrix interference evaluation

	% Matrix interference	
	Analyte	Internal standard
M1	6.7	0.12
M2	5.6	0.12
M3	14.7	0.15
M4	9.4	0.14
M5	13.4	0.14
M6	7.3	0.14

CONCLUSION

- **High throughput quantification of Dypiridamole in cell protein precipitated solution performed in 5 sec per sample using LDTD-MS/MS.**
- **Good linearity with coefficients higher than 0.995**
- **Intra and Inter-assay precision between 3.7 to 9.7% (%RSD)**
- **Intra and Inter-assay accuracy between 92.8 to 103.5% (%Nom)**
- **Matrix interference and carry over were lower than 20% at LLOQ concentration and lower than 5% at internal standard.**