Phytronix

Application Note 0710

LDTD-MS/MS Analysis in 9 seconds : Quantification of Mifepristone in Mouse Plasma

Pierre Picard and Patrice Tremblay

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Overview

- High-throughput determination of mifepristone and two mifepristone analogues in mouse plasma is performed by LDTD-MS/MS;
- Calibration range from 10 to 2000 ng/mL with $r^2 > 0.995$;
- Sample-to-sample run time of 9 seconds;
- Accuracy within 95.1 and 113.4 %;
- Mean precision of 4.9 ± 3.2 %;
- Excellent method selectivity from blank analysis.

Instrumentation

- Phytronix Technologies LDTD ion source (model T-960);
- Thermo Fisher Scientific TSQ® Quantum™ Ultra AM mass spectrometer.

LDTD ionization process

The LDTD ion source uses an infrared laser diode to desorbs sample that have been dried onto a well of a LazWell™ (96-well plate). The desorbed gas phase molecules are carried into a corona discharge region to undergo APCI, then they are transferred directly into the mass spectrometer for detection.

Samples Preparation

Mouse herapin plasma was spiked with mifepristone and two mifepristone analogues and with ISTD (deuterated drugs). The drugs were extracted with MTBE and Hexane (1:1 v/v) and reconstitute into a water:acetonitrile:formic acid solution (75:25:0.1 v/v/v). A volume of 2.0 µL was manually transferred into a well of a LazWell™ and was allowed to dry at room temperature.

Results and Discussion

Calibration Curves

Quantitative determination of mifepristone and two mifepristone analogues in mouse plasma can be achieved over a nominal concentration range of 10 to 2000 ng/mL (**Figure 1**). An excellent linearity is obtained over the concentration range ($R^2 > 0.995$).

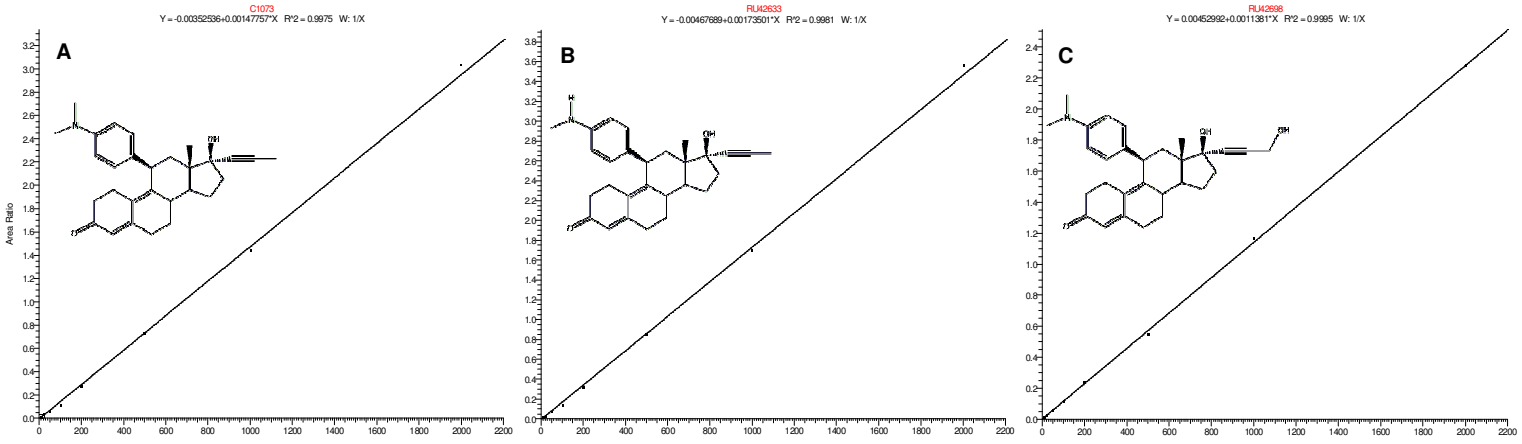

Figure 1 Calibration curve of A) mifepristone and two mifepristone analogues, B) MifA-1 and C) MifA-2 in mouse plasma.

Accuracy and Precision

Quality control samples were analyzed to evaluate the LDTD-MS/MS method accuracy and precision. The accuracy was evaluated to be within 95.1 and 113.4 % and the mean precision was 4.9 ± 3.2 % (**Table 1**)

Table 1 Within-run accuracy and precision for mifepristone and mifepristone analogues.

	Mifepristone		
	QC1	***QC2***	***QC3***
Nominal conc. (ng/mL)	30	300	1600
N	3	3	3
Mean (ng/mL)	30.6	287	1634
RSD (%)	7.0	6.2	1.9
% Nominal conc.	101.8	95.8	102.1
	MifA-1		
	QC1	***QC2***	***QC3***
Nominal conc. (ng/mL)	30	300	1600
N	3	3	3
Mean (ng/mL)	29.0	285	1646
RSD (%)	3.8	3.7	5.8
% Nominal conc.	96.7	95.1	102.9
	MifA-2		
	QC1	***QC2***	***QC3***
Nominal conc. (ng/mL)	30	300	1600
N	3	3	3
Mean (ng/mL)	30.5	326	1815
RSD (%)	1.9	11.7	2.1
% Nominal conc.	101.6	109.0	113.4

Mifepristone LDTD Desorption Profile

The LDTD allows fast mifepristone thermal desorption (**Figure 2**). The blank samples (signal intensity of 557) allows a LOD of 0.9 ng/mL.

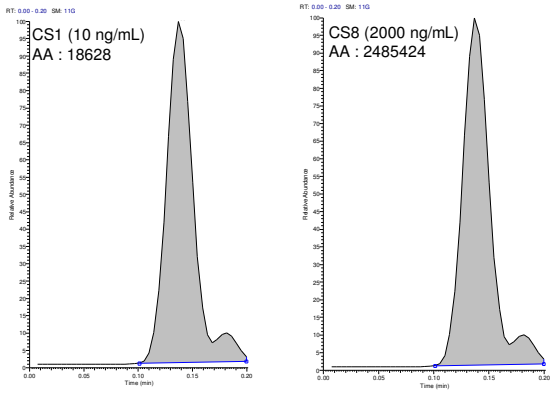

Figure 2 Desorption profile for 2 mifepristone standards.

Method Selectivity

The selectivity was evaluated from blank samples analysis. The blank signals were interfering from 3.3 to 8.1 % on CS1 samples (10 ng/mL). The interference on the internal standards were lower then 0.05 %. Even without chromatographic separation, LDTD-MS/MS analysis shows an excellent method selectivity.

MS Parameters

APCI (+)	
Collision gas pressure	1.5 mTorr (Argon)
Collision energy (Mifepristone, MifA-1, MifA-2)	18, 20, 25 V
Tube lens (Mifepristone, MifA-1, MifA-2)	77, 89, 112 V
Scan time	0.030 s
Needle voltage	5000 V
Q1 width	0.70 amu
Q3 width	0.70 amu
Mifepristone SRM transition	430.14 → 372.25 amu
Mifepristone-d4 SRM transition	434.15 → 374.25 amu
MifA-1 SRM transition	416.12 → 358.04 amu
MifA-1-d4 SRM transition	420.12 → 360.04 amu
MifA-2 SRM transition	446.14 → 388.22 amu
MifA-2-d4 SRM transition	450.15 → 390.22 amu

LDTD Parameters

Laser power pattern	0 to 60 % in 1.0 s Hold at 60 % for 2.0 s
Carrier gas flow	3.0 L/min (Air)

Conclusions

LDTD-MS/MS allows high-throughput quantification of mifepristone and two mifepristone analogues with a sample-to-sample run time of 9 seconds. LDTD-MS/MS shows no matrix effect and no observed carryover and a excellent method selectivity.

High-throughput analysis with excellent linearity, accuracy and precision can be achieved using LDTD as ion source in mass spectrometry.