Application Note 0709

Midazolam and 1-Hydroxymidazolam in Human Plasma : LDTD-MS/MS Analysis in 8 seconds

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Overview

- High-throughput determination of midazolam and 1-hydroxymidazolam in human plasma is performed by LDTD-MS/MS;
- Calibration range from 0.5 to 250 ng/mL with $r^2 > 0.997$;
- Sample-to-sample run time of 8 seconds;
- Accuracy within 94.9 and 112 %;
- Precision within 1.3 and 10.9 %.

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

Human plasma was spiked with midazolam and 1-hydroxymidazolam and with ISTD (deuterated midazolam and 1-hydroxymidazolam). The drugs were extracted with MTBE and hexane (3:1 v/v) and reconstitute into a water:acetonitrile solution (75:25 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 midazolam and 1-hydroxymidazolam in human plasma can be achieved over a nominal concentration range of 0.5 to 250 ng/mL (**Figure 1 and 2**). An excellent linearity is obtained over the concentration range ($R^2 > 0.997$).

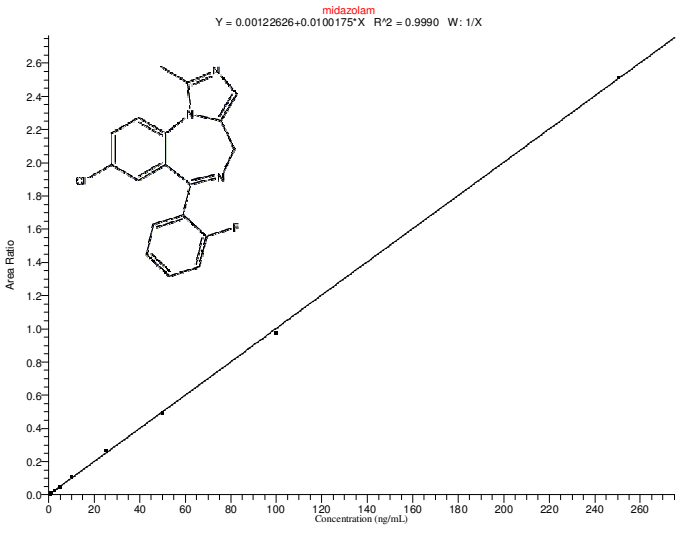

Figure 1 Calibration curve of midazolam in human plasma.

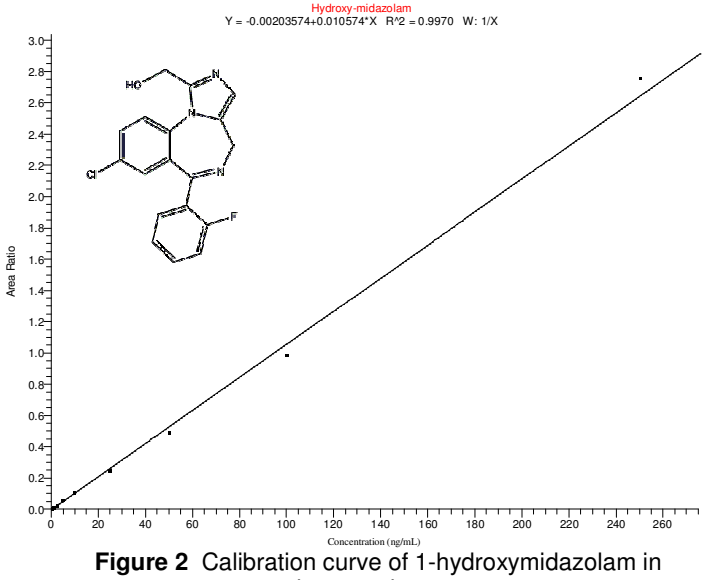

Figure 2 Calibration curve of 1-hydroxymidazolam in human plasma.

Accuracy and Precision

Quality control samples were analyzed in triplicates to evaluate the LDTD-MS/MS method accuracy and precision. The accuracy was evaluated to be within 94.9 and 112.2 % and the precision was within 1.3 and 10.9 % (**Table 1**)

Table 1 Within-run accuracy and precision for midazolam and 1-hydroxymidazolam.

	Midazolam		
	QC1	***QC2***	***QC3***
Nominal conc. (ng/mL)	1.5	15	200
N	3	3	3
Mean (ng/mL)	1.42	14.3	190.0
RSD (%)	10.9	6.0	1.3
% Nominal conc.	94.9	95.0	95.0
	1-Hydrozymidazolam		
	QC1	***QC2***	***QC3***
Nominal conc. (ng/mL)	1.5	15	200
N	3	3	3
Mean (ng/mL)	1.68	15.7	191.6
RSD (%)	3.8	4.3	1.6
% Nominal conc.	112.2	104.8	95.8

Midazolam and 1-Hydroxymidazolam LDTD Desorption Profile

The LDTD allows fast midazolam and 1-hydroxymidazolam thermal desorption (**Figure 3**). Blank sample signals allo a LOQ of 0.1 ng/mL for midazolam and of 0.8 ng/mL for 1-hydroxymidazolam.

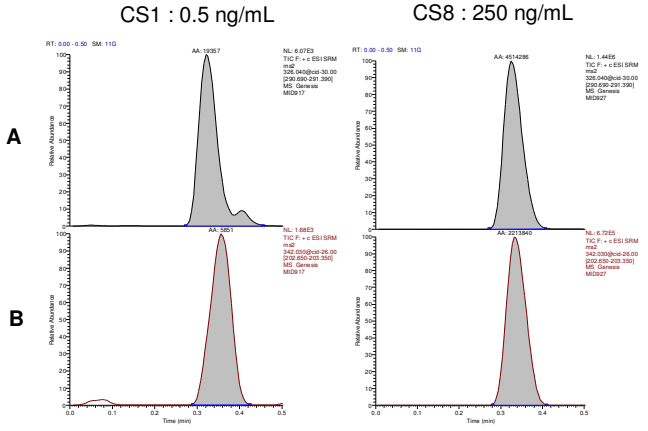

Figure 3 Desorption profile for 2 calibration standards for A) midazolam, and B) 1-hydroxymidazolam.

1-Hydroxymidazolam Back-conversion Evaluation

1-Hydroxymidazolam may back-convert into midazolam during the thermal desorption and/or the ionization process. To evaluate this back-conversion, 5 ng of 1-hydroxymidazolam was analyzed in LDTD-MS/MS and both midazolam and 1-hydroxymidazolam SRM transitions were monitored. The back-conversion of 1-hydroxymidazolam into midazolam was evaluated to be 0.18 % which does not affect midazolam quantification at plasma concentration.

MS Parameters

APCI (+)	
Collision gas pressure	1.5 mTorr (Argon)
Collision energy	27 V
Tube lens	77 V
Scan time	0.050 s
Needle voltage	5000 V
Q1 width	0.70 amu
Q3 width	0.70 amu
Midazolam SRM transition	326.04 → 291.04 amu
Midazolam-d4 SRM transition	330.00 → 295.00 amu
1-OH-midazolam SRM transition	342.03 → 203.00 amu
1-OH-midazolam-d4 SRM transition	346.00 → 203.00 amu

LDTD Parameters

Laser power pattern	0 to 40 % in 2.0 s Hold at 40 % for 2.0 s
Carrier gas flow	3.0 L/min (Air)

Conclusions

LDTD-MS/MS allows high-throughput quantification of midazolam and 1-hydroxymidazolam with a sample-to-sample run time of 8 seconds. Low back-conversion (0.8 %) was observed on 1-hydroxymidazolam.

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