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Quantitation of Midazolam and 1'-Hydroxymidazolam in Human Plasma Using API-4000 LC-MS/MS Systems with Higher Specificity and Lower Background Noise

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Overview

Using liquid-liquid extraction procedure, a sensitive and specific liquid chromatographic-tandem mass spectrometric (LC/MS/MS) method capable of quantifying Midazolam and 1'-Hydroxymidazolam in human plasma is described.

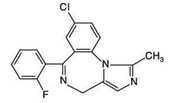
In this method, the drug was extracted from a 0.1 mL of human plasma using simple extraction method. Separation was performed on a reverse phase C8 column. Detection was achieved using a AB/SCIEX API-4000 system in the positive ion mode along with multiple reaction monitoring (MRM). The lower limit of quantitation was 0.5 ng/mL .

This method has been successfully applied to clinical pharmacokinetic studies.

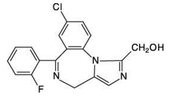
Introduction

Midazolam is a short-acting drug in the bedzodiazepine derivative. Benzodiazepines belong to the group of medicines called central nervous system (CNS) depressants, which are medicines that slow down the nervous system. In recent years, some analytical methods have been developed for pharmacokinetic studies, but most LC-MS/MS methods have issues due to lower specificity, higher background noise, and poor chromatograms. In this study, the positive ESI multiple reaction monitoring (MRM) mode of API-4000 LC-MS/MS Systems was used to measure Midazolam and its metabolite, 1'-Hydroxymidazolam in human K_2 EDTA plasma with higher specificity and lower background noise.

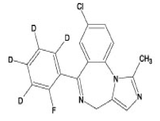
Structure



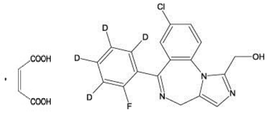
Midazolam



1'-Hydroxy Midazolam



Midazolam-d4 (IS-1)



1'-Hydroxy Midazolam-d4(IS-2)

Methods

Sample Preparation:

Plasma samples were extracted by using the 100-µL aliquot of plasma. After extraction, the extracts must be centrifuged at 14,000 rpm for about at least 10 minutes. The extract was then transferred to LC vials for LC-MS/MS or stay in the 96-well plate for the analysis.

Liquid Chromatography:

Pump: Shimadzu UFLC LC-20A
Autosampler: Shimadzu UFLC SIL-20AC$_{XR}$
System Controller: Shimadzu CBM-20A
Analytical Column: C8 column, 2.0 x 50 mm, 5 µm
Gradient: The analytes were eluted using a gradient of mobile phase A (0.1% formic acid) and mobile phase B (0.1% formic acid in methanol) from 25% to 95% mobile phase B in 3.0 min.
Injection Volume: 5 µL

Mass Spectrometry:

MS System: AB/Sciex API-4000
Condition: LC/(+)ESI-MS/MS,
MRM transition:
Midazolam: 326.2→291.2
1'-Hydroxymidazolam: 342.2→203.2

Results and Discussion

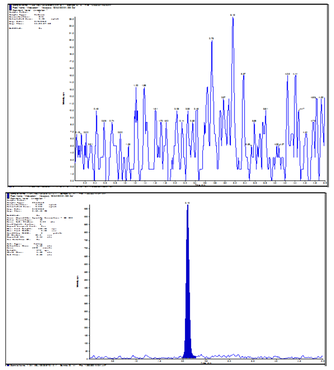


Figure 1. Ion chromatograms of blank plasma (Upper), and 0.5 ng/mL Midazolam extracted from plasma (Lower) with Unit Resolution

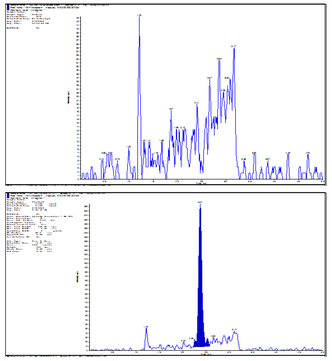


Figure 2. Ion chromatograms of blank plasma (Upper), and 0.5 ng/mL 1'-OH Midazolam extracted from plasma (Lower) with Unit Resolution

Table I. Validation Data Summary for Midazolam

Calibration Range		0.5 to 500 ng/mL		
Correlation coefficient(r^2, mean)		0.9989		
Accuracy & Precision			Accuracy	Precision
	QC	Conc. (ng/mL)	RE%	CV%
Inter-Batch (n=18)	LLOQ	0.5	-1.6	3.7
	Low	1.5	-3.3	4.3
	Medium	40	-0.8	2.6
	High	400	0.5	2.5
	Compared with Nominal Value (%)			
Method Recovery	>94.2			
				Accuracy
		Condition		RE%
Freeze/Thaw		3 Cycles, <-70 ºC		<1.2
Bench-Top		4 hrs, Room Temperature		<1.2
Autosampler Extract Stability		26 hrs, Room Temperature		<1.3
Long-Term Storage Stability		32 Days, <-70 ºC		<4.1

Table II. Validation Data Summary for 1'-OH Midazolam

Calibration Range		0.5 to 500 ng/mL		
Correlation coefficient(r^2, mean)		0.9983		
Accuracy & Precision			Accuracy	Precision
	QC	Conc. (ng/mL)	RE%	CV%
Inter-Batch (n=18)	LLOQ	0.5	-1.2	11.3
	Low	1.5	-6.0	5.4
	Medium	40	0.3	2.6
	High	400	1.0	2.7
	Compared with Nominal Value (%)			
Method Recovery	>96.4			
				Accuracy
		Condition		RE%
Freeze/Thaw		3 Cycles, <-70 ºC		<4.5
Bench-Top		4 hrs, Room Temperature		<1.1
Autosampler Extract Stability		26 hrs, Room Temperature		<2.6
Long-Term Storage Stability		32 Days, <-70 ºC		<6.0

• Excellent linearity was obtained with a correlation coefficient ≥ 0.9977 for Midazolam and ≥ 0.9987 for 1'-Hydroxymidazolam. The high dynamic calibration range was reached due to eliminated background noise. (Table I, II Figures 1 to 3).

• For Midazolam, including LLOQ, the inter-day CV ranged from 2.5% to 4.3% and the biases of the means ranged from -3.3% to 0.5%. For 1'-Hydroxymidazolam, including LLOQ, the inter-day CV ranged from 2.6% to 11.3% and the biases of the means ranged from -6.0% to 1.0%. These results also indicate that the liquid-liquid extraction method is more suitable than protein precipitation extraction method for Midazolam and 1'-Hydroxymidazolam analysis in human K_2 EDTA plasma.

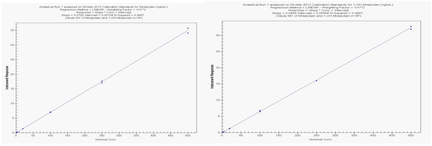


Figure 3. Typical Calibration Curve of Testosterone (Left) and 1'-Hydroxymidazolam (Right) in Human Plasma

Conclusions

A rapid, simple and specific LC-MS/MS method has been developed and validated for quantifying Midazolam and 1'-OH Midazolam with a lower limit of quantitation of 0.5 ng/mL from a 0.1 mL plasma sample.