Development of an LC-MS/MS Bioanalytical Method for Midazolam and 1-OH Midazolam

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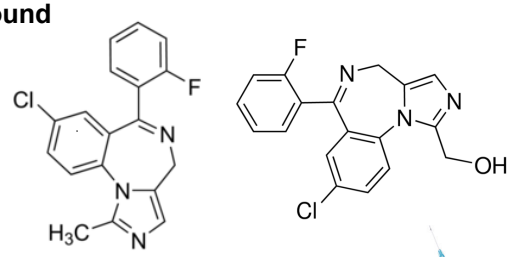
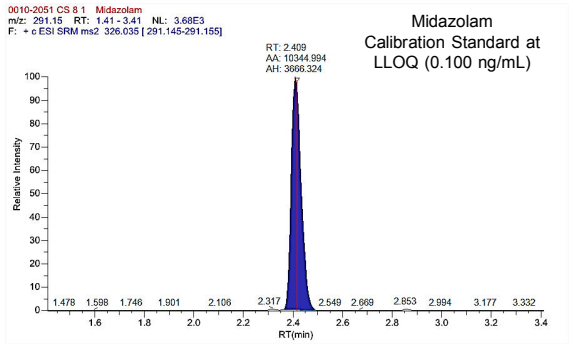
Background

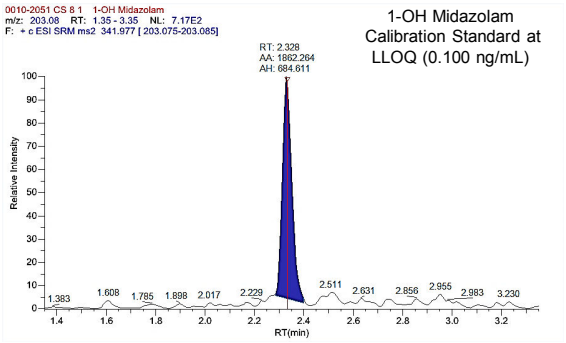


- Classified as a benzodiazepine
- Given intravenously to induce general anesthesia
- Mediated through the inhibitory neurotransmitter gamma-aminobutyric acid (GABA)
- Applications include preoperative sedation and sedation during diagnostic, therapeutic, and endoscopic procedures

Chromatograms

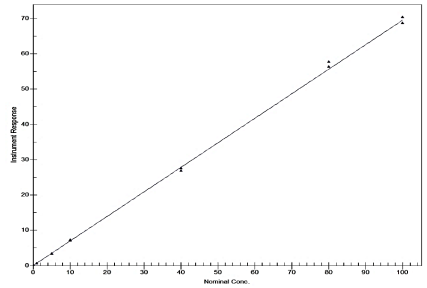


Midazolam
Calibration Standard at
LLOQ (0.100 ng/mL)

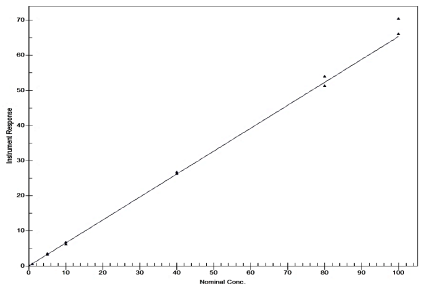


1-OH Midazolam
Calibration Standard at
LLOQ (0.100 ng/mL)

Calibration Curves



Midazolam
Linear regression
1/X² Weighting
R²:0.9981



1-OH Midazolam
Linear regression
1/X² Weighting
R²:0.9961

Sample Preparation Method

- Validated range: 0.100-100 ng/mL in human plasma
- Working solutions: prepared in water:methanol (50:50)
- Liquid-liquid extraction (LLE) procedure
  - Sample volume: 100 µL
  - A 25.0 µL aliquot of Internal standard was added to the samples
  - A 100 µL aliquot of water was added to the samples
  - A 600 µL aliquot of methyl tert-butyl ether:dichloromethane (75:25) was added to the samples
  - The samples were capped, shaken, vortexed, and centrifuged
  - A 500 µL aliquot of the organic layer was transferred and evaporated to dryness
  - Samples were reconstituted in 100 µL of water:acetonitrile:formic acid (80:20:0.1)

Note: A Tomtec liquid handling robot was used for extraction solvent addition and sample transfer

Instrumental Analysis Method

Waters™ Acquity UPLC		Thermo™ TSQ Vantage MS	
Run Time:	3.5 min	Ion Source:	HESI
Column Temp.:	30 °C	Spray Voltage:	2000
Autosampler Temp.:	7.5 °C	Ion Transfer Tube Temp:	225 °C
Flow Rate:	0.3 mL/min	Vaporizer Temp:	400 °C
Mobile Phases:	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile	Sheath Gas	40
LC Program	20 to 40% MP B over 1 min	Aux Gas	20
Analytical Column:	Waters™ Acquity UPLC® BEH C18,1.7 µm, 2.1 x 50 mm	Resolution	Unit/Unit

SRM Table

Compound	Polarity	Precursor (m/z)	Product (m/z)	Collision Energy (V)	S Lens (V)
Midazolam	Positive, +1	326.07	291.16	26	143
Midazolam-D4	Positive, +1	332.09	295.18	26	143
1-OH midazolam	Positive, +1	342.06	203.07	26	117
1-OH midazolam-D4	Positive, +1	348.08	205.07	26	117

Inter-Assay Performance of QC Samples

Midazolam

Summary Statistics	Run ID	LLOQ VS 0.100 ng/mL	LOW VS 0.300 ng/mL	MIDDLE VS 7.50 ng/mL	HIGH VS 75.0 ng/mL
Mean Concentration Found	5, 6, and 7	0.101	0.309	7.68	76.8
Inter-run SD		0.00679	0.0087	0.128	2.15
Inter-run % CV		6.7	2.8	1.7	2.8
Inter-run %Bias		1	3	2.4	2.4
n		24	24	24	24

1-OH Midazolam

Summary Statistics	Run ID	LLOQ VS 0.100 ng/mL	LOW VS 0.300 ng/mL	MIDDLE VS 7.50 ng/mL	HIGH VS 75.0 ng/mL
Mean Concentration Found	5, 6, and 7	0.101	0.316	7.67	77.1
Inter-run SD		0.0112	0.0191	0.236	1.8
Inter-run % CV		11.1	6	3.1	2.3
Inter-run %Bias		1	5.3	2.3	2.8
n		24	24	24	24

Validation Results

- Three core runs met acceptance criteria for precision and accuracy.
- All selectivity results (unspiked and spiked) were well within acceptance criteria.
- Suitable for lipemic and hemolyzed samples.
- Recovery not lower than 94.2% achieved across both analytes and internal standards.
- No impact or suppression effects observed in matrix factor samples.
- Excellent stability (bench top, freeze/thaw, frozen storage, extract, and whole blood) established
- More than 2 days of reinjection reproducibility established.
- Suitable for many samples (2 plate runs are validated).

Conclusions

A sensitive and specific LC-MS/MS assay for the measurement of Midazolam and 1-OH midazolam in human plasma has been developed and validated.

This robust method features simple LLE sample preparation, short run time, and high recovery.

The method is precise and accurate across a 1000-fold range and has been employed for regulated analysis of hundreds of clinical samples with 100% ISR passing rate.