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Application News

No. AD-0069

LCMS-8040 UFMS

Fast LC/MS/MS Method for Quantitative Determination of Omeprazole in Human Plasma

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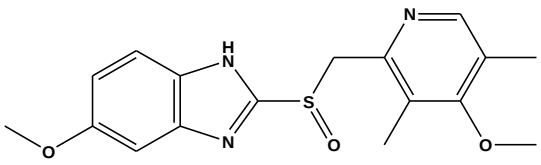
Abstract

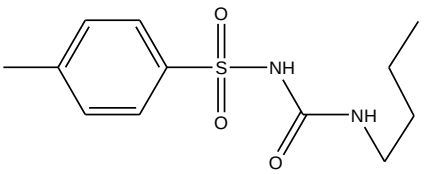
A sensitive and a fast LC/MS/MS method for quantification of Omeprazole in human plasma using UHPLC NEXERA coupled to LCMS-8040 Triple Quadrupole Mass Spectrometer was described. A simple liquid-liquid extraction method for extraction of Omeprazole and internal standard (Tolbutamide) from the biological matrix was employed. The new advanced LCMS-8040 enabled the quantification of Omeprazole from human plasma samples over a concentration range of 1.0 (LLOQ) to 2000ng/mL. The constructed calibration curve was linear with a regression of >0.99.

❑ Introduction

Omeprazole is a well-studied proton pump inhibitor, which inhibits the gastric parietal cell proton pump, dose-dependently reducing basal and stimulated gastric secretion and raising intragastric pH. It belongs to a class of anti-secretary compound used in treatment of peptic ulcer, gastro esophageal reflux, dyspepsia. Omeprazole is one of the most widely prescribed drugs internationally and is available over the counter in some countries. The chemical name for Omeprazole is (RS) -5-methoxy-2-((4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl)-5-methhoxy-1H-benzo[d]imidazole. For routine analysis of bio-fluids, simple sample preparation protocols that are sensitive and specific are preferred. LC/MS/Ms is then the method of choice for analytes extracted from biological matrices. In this note, a simple, fast and a sensitive method for quantitative determination of Omeprazole in human plasma with Tolbutamide as internal standard is described using the UHPLC Nexera coupled to LCMS-8040 instrument.



Omeprazole



Tolbutamide (IS)

Figure-1 : Chemical structure of Omeprazole and Tolbutamide (IS)

❑ Experimental

Preparation of Aqueous standards: A 1 mg/mL Stock solution of internal standard (IS) was prepared in acetonitrile. This solution was serially diluted using acetonitrile to obtain a working solution of internal standard containing 300µg/mL of Tolbutamide (IS). Similarly a 1mg/mL stock solution of Omeprazole was prepared using Methanol as diluent. This solution was further serially diluted using Methanol to obtain aqueous standards containing Omeprazole of concentration 10.0, 20.0, 200.0, 1000, 5000, 7500, 15000 and 20000 ng/mL respectively.

Preparation of Plasma calibration standards (CC): 180µL of human plasma was spiked with 20 µL of each aqueous Omeprazole standard solution and vortexed for 30 seconds to obtain plasma calibration standard whose concentration ranged from 1.0 – 2000 ng/mL. Each of these samples were then extracted according to the procedure as described under sample preparation.

Preparation of plasma quality control standards (QC): The Quality control standard solutions were prepared at three intermediate concentrations of that of CC standards namely 3.0, 900.0 and 1800.0 ng/mL (LQC, MQC and HQC respectively). Six individual preparations of each of the QC standards were prepared to evaluate precision and recovery. Each of these sample preparation were then extracted according to the procedure as described under

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sample preparation.

Sample preparation: 200 µL of Sodium bicarbonate buffer (pH 10.5) was added to each of the plasma sample taken in a centrifuge tube and vortexed briefly for a period of 60s. A 600 µL of Ethyl acetate was then added to each of the tubes and mixed for a period of 5 minutes. The samples were then centrifuged using a *minispin* at 10000 rpm for 10 minutes. A 100 µL of the upper layer was then mixed with 200 µL of acetonitrile and injected in to the LC/MS/MS system.

Table-1 : Analytical conditions

Column	: Capcell pak C18, 50 x 2.0mm, 3.0µm		
Mobile phase-A	: 0.02m ammonium formate in water pH-3.0		
Mobile phase-B	: Acetonitrile: Methanol (50:50) v/v		
Gradient (%B/T)	: 5/0.01, 95/2.00, 5/2.01, & 5/3.5		
Flow rate	: 800 µL/min	DL temp	: 220 ^{0}C
Column temp	: 35 ^{0}C	Heat block	: 275 ^{0}C
Drying gas	: 15 L/min	Interface	: ESI
Nebulizing gas	: 3.0 L/min	Interface volt	: 4.5 kV
For Omeprazole			
MRM	: 346.00 → 198.10	Polarity	: Positive
Dwell time	: 100 ms	CE	: - 13.0V
Q1 pre-bias	: - 24.0V	Q3 pre-bias	: - 38.0V
For Tolbutamide			
MRM	: 269.00 → 170.10	Polarity	: Negative
Dwell time	: 100 ms	CE	: 17.0V
Q1 pre-bias	: 28.0V	Q3 pre-bias	: 34.0V

The LC-MS conditions are as summarized as in Table-1. Precursor ions of Omeprazole and Tolbutamide (IS) were determined by injecting a solution containing these compounds in the Q1 scan mode. Under these conditions, the analyte and the IS yielded predominantly the quasi molecular ions of m/z 346 and m/z 269 respectively. Each of these precursor ions was subjected to collision induced dissociation (CID) in order to generate product ions. This operation was done automatically by the use of SSS (Synchronized Survey Scan) function in the software to obtain optimized parameters. Based on this, the ion transitions of m/z 346.00 → 198.10 and m/z 269.00 → 170.10 (Figure-2) were used in MRM mode for Omeprazole and Tolbutamide (IS) respectively.

❑ Results and Discussion

LLOQ

The concentration of Omeprazole at lower limit of quantitation (LLOQ) was determined to be 1.0 ng/mL. This was confirmed from the coefficient of variance (CV) being less than 20% for the six replicate injections of Omeprazole at this concentration. The overlay mass chromatograms corresponding to Omeprazole is as presented in Figure-3.

Linearity

The CC standards were used to construct a calibration curve by plotting the area ratio of Omeprazole with respect to IS versus the concentration of CC standards. Linear curve fit type was used and weighted ($1/x^2$). A linear dynamic range of 1.0 to 2000.0 ng/mL was achieved for

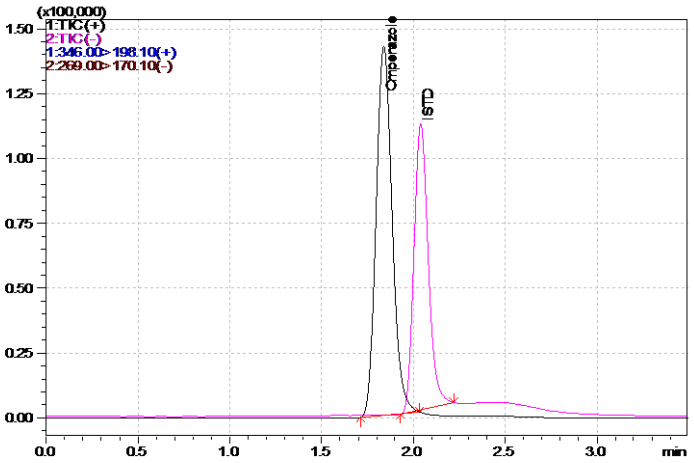

Figure-2 : MRM mass chromatogram of Omeprazole at MQC

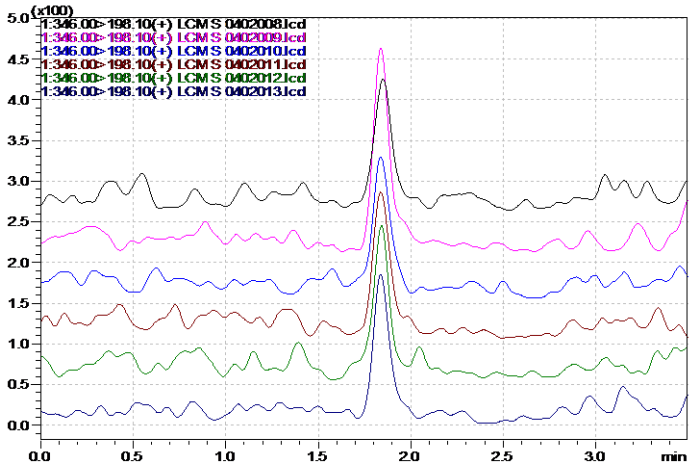

Figure-3 : Overlay chromatograms of Omeprazole at LLOQ

Omeprazole with a R^2 value of 0.99987 .

Figure-4 shows a representative calibration curve of Omeprazole in plasma using Tolbutamide as internal standard.

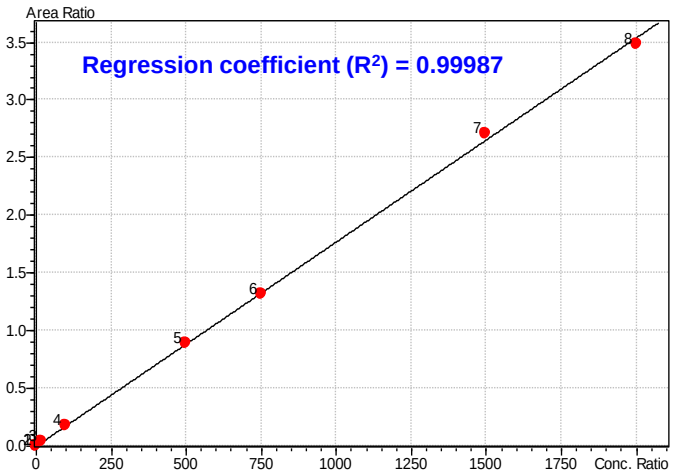

Figure-4 : Calibration curve of Omeprazole

Precision & Accuracy of QC samples

Low, middle and high QC samples containing Omeprazole were prepared at concentrations of 3, 900 and 1800 ng/mL in plasma. The precision (%CV, n=6) for the QCs for Omeprazole varied from 3.1 to 12.4% and accuracy from 80.0 to 113.3% of the nominal value (Table-2).

Table 2: Precision and accuracy of Omeprazole in QC samples

Nominal Conc. (ng/mL)	Measured conc. (ng/mL)	Accuracy*	Precision (n=6)
3.0	2.7	90.0	12.4
	2.5	83.3	
	3.4	113.3	
	3.2	106.7	
	2.4	80.0	
	2.7	90.0	
900.0	826.0	91.8	3.1
	802.9	89.2	
	771.4	85.7	
	842.7	93.6	
	828.2	92.0	
	824.5	91.6	
1800.0	1704.1	94.5	4.9
	1514.1	84.1	
	1536.5	85.4	
	1631.0	90.6	
	1554.2	86.3	
	1514.9	84.2	

* expressed as Bias = (mean concentration/nominal concentration) x 100

Table 3: Recovery of Omeprazole in QC samples

QC sample	Number of Preparations	% Recovery
		Omeprazole
LQC	1	103.9
	2	89.2
	3	114.4
	4	107.3
	5	103.0
	6	99.0
MQC	1	96.8
	2	95.8
	3	95.9
	4	96.5
	5	97.2
	6	98.4
HQC	1	95.0
	2	92.1
	3	91.7
	4	96.7
	5	93.8
	6	86.0

Recovery of QC samples

The recovery of Omeprazole was calculated by comparing the peak area obtained for QC samples that were subjected to extraction procedure with those obtained from blank plasma extracts that were spiked post extraction to the same nominal concentrations. Good recoveries were obtained (Table-3) for Omeprazole demonstrating the efficiency of analyte extraction in the presence of biological matrix.

❑ Conclusion

A simple, high throughput LC-MS/MS method for quantitative determination of Omeprazole in human plasma was developed. The LLOQ of the method using a 180 µL of plasma was determined as 1.0ng/mL. The linear dynamic range of the calibration curve was 1.0 – 2000 ng/mL with a regression of R^2 = 0.99987. Good recoveries (between 86.0 to 114.4%) were obtained were obtained at all the three levels of QC samples with repeatability .

❑ Reference

J. De Smet et al. J.Sep. Sci. 2010, 33, 939-947

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