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Application News

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LCMS-8040 UFMS

A Fast LC/MS/MS method for Determination of Telmisartan in Human Plasma by LC-MS-MS

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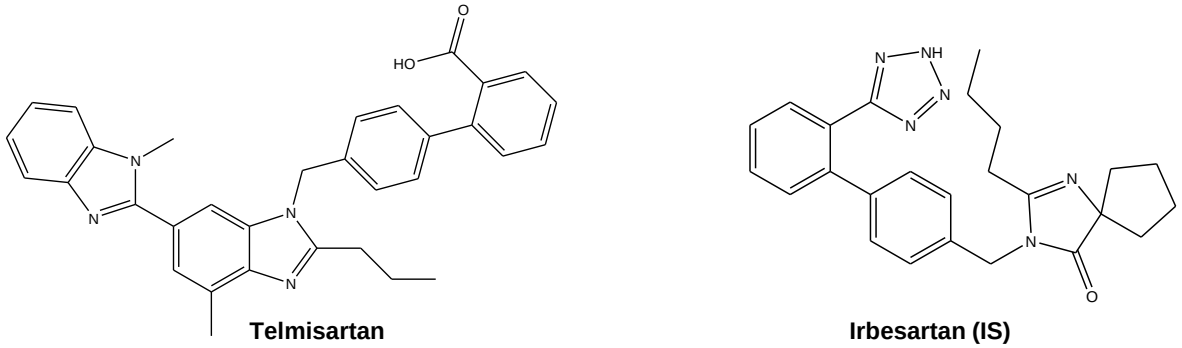
Abstract

A fast LC/MS/MS method for quantitative determination of Telmisartan in human plasma using UHPLC Nexera coupled to LCMS-8040 Triple Quadrupole Mass Spectrometer was described. A simple protein precipitation method for extraction of Telmisartan with internal standard (Irbesartan) from the biological matrix was employed. The new advanced LCMS-8040 enabled the quantification of Telmisartan from human plasma samples over a concentration range of 4.0 (LLOQ) to 2000 ng/mL. The constructed calibration curve was linear with a regression of coefficient more than 0.99.

❑ Introduction

Telmisartan (2-(4-{[4-methyl-6-(1-methyl-1*H*-1,3-benzodiazol-2-yl)-2-propyl-1*H*-1,3-benzodiazol-1-yl]methyl}phenyl)benzoic acid) is an angiotensin II receptor antagonist widely used in treatment of hypertension. It undergoes minimal bio-transformation in the liver to form the inactive Telmisartan-1-o-acylglucoronide as its principal metabolite. The long half-life and selectivity of Telmisartan for angiotensin II receptors allows once daily dosing with minimal side effects. Liquid chromatography with tandem mass spectrometry (LC/MS/MS) has been widely employed for bioassays of drugs. In this study, a simple and fast method for quantitative determination of Telmisartan in human plasma with Irbesartan as internal standard is described using the UHPLC Nexera coupled to LCMS-8040 instrument.



Telmisartan **Irbesartan (IS)**

Figure 1 : Chemical structure of Telmisartan and Irbesartan (IS)

❑ Experimental

Preparation of Aqueous Standards: Stock solution at concentration of 1 mg/mL of Telmisartan was prepared in acetonitrile:water (80:20 v/v) mixture. This solution was further serially diluted using acetonitrile:water (80:20 v/v) mixture to obtain aqueous standards containing Telmisartan at the concentration of 40, 80, 200, 400, 800, 2000, 4000, 8000, 12000, 16000 and 20000 ng/mL respectively. Similarly, a stock solution of Irbesartan, which is used as the internal standard (IS) was prepared at the concentration of 1 mg/mL in acetonitrile:water (80:20 v/v) mixture. This solution was further diluted to obtain internal standard working solution (ISTD) at a concentration of 3000 ng/mL.

Preparation of Plasma Calibration Standards (CC): 180 µL of human plasma was spiked with 20 µL of each aqueous Telmisartan standard solution and 20 µL of ISTD solution, vortexed for 30 seconds to obtain plasma calibration standard whose concentration ranged from 4.0 – 2000 ng/mL. Each of these samples was 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 CC standards namely 12.0, 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 was then extracted according to the procedure as described under sample preparation.

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Sample Preparation: A simple protein precipitation method for extraction of Telmisartan and the IS from the plasma matrix was employed. All plasma samples were treated with 1000 µL of ice-cold acetonitrile and vortexed for 5 minutes. The samples were then centrifuged at 10,000 rpm for 10 minutes. The supernatant layer was directly taken for injection into the LC/MS/MS system.

Table 1 : Analytical conditions

Column	: Zorbax Eclipse Plus C18, 100 x 2.1mm, 1.8 µm		
Mobile phase-A	: 0.02M Ammonium acetate in water		
Mobile phase-B	: Acetonitrile		
Isocratic	: A:B (60:40 v/v)		
Flow rate	: 600 µL/min	DL temp	: 250 ^{0}C
Column temp	: 50 ^{0}C	Heat block	: 300 ^{0}C
Drying gas	: 15 L/min	Interface	: ESI
Nebulizing gas	: 3.0 L/min	Interface volt	: 4.5 kV

For Telmisartan

MRM	: 515.00 → 276.10	Polarity	: Positive
Dwell time	: 100 ms	CE	: -49.0 V
Q1 pre-bias	: -40.0 V	Q3 pre-bias	: -26.0 V

For Irbesartan (IS)

MRM	: 429.00 → 207.00	Polarity	: Positive
Dwell time	: 100 ms	CE	: -24.0 V
Q1 pre-bias	: -32.0 V	Q3 pre-bias	: -40.0 V

The LC-MS conditions are summarized in Table 1. Precursor ions of Telmisartan and Irbesartan (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 515 and m/z 429 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 515.00 → 276.00 and m/z 429.00 → 207.00 (Figure 4) were used in MRM mode for Telmisartan and Irbesartan (IS) respectively.

❑ Results and Discussion

LLOQ

The concentration of Telmisartan at lower limit of quantitation (LLOQ) was determined to be 4.0 ng/mL. This was confirmed from the coefficient of variance (CV) being less than 20% for the six replicate injections of Telmisartan at this concentration. The overlay mass chromatograms corresponding to Telmisartan at its LLOQ and the blank is presented in Figure 3.

Linearity

A linear dynamic range of 4.0 to 2000.0 ng/mL was achieved for Telmisartan with R^2 value of 0.9970. The CC standards were used to construct a calibration curve by plotting the area ratio of Telmisartan with respect to IS versus the concentration of CC standards. Linear curve fit type was used and weighted ($1/x^2$). Figure 2 shows a representative calibration curve of Telmisartan in plasma using Irbesartan as internal standard.

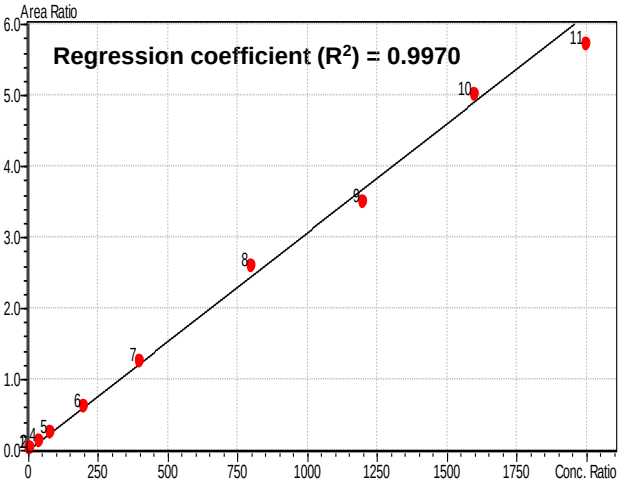

Figure 2 : Calibration curve of Telmisartan

The calculated concentrations for all the CC standards were within ±10% of the nominal value as determined by bias calculation (Table 2).

Table 2: Accuracy of Telmisartan in CC samples

Nominal Concentration (ng/mL)	Measured Concentration (ng/mL)	Accuracy*
4.0	3.9	98.6
8.0	8.4	105.5
20.0	18.1	90.5
40.0	41.9	104.8
80.0	79.7	99.6
200.0	203.0	101.5
400.0	404.8	101.2
800.0	848.5	106.1
1200.0	1149.1	95.8
1600.0	1643.7	102.7
2000.0	1873.4	93.7

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

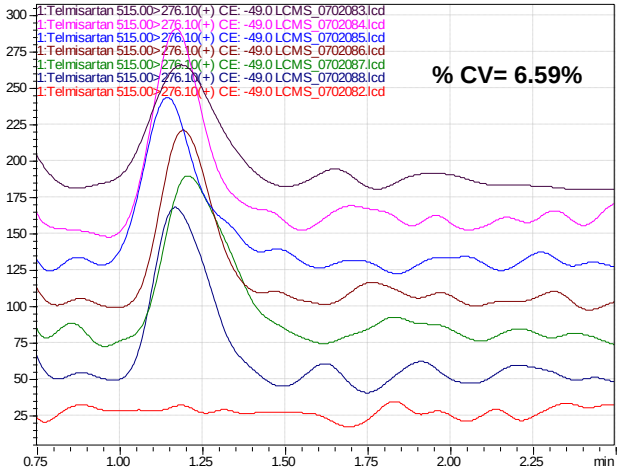

Figure 3 : Overlay chromatograms of Telmisartan at LLOQ conc.with blank (bottom)

Precision & Accuracy of QC Samples

Low, middle and high QC samples containing Telmisartan were prepared at concentrations of 12, 900 and 1800 ng/mL in plasma. The precision (%CV, n=6) of the QCs for Telmisartan varied from 3.6 to 5.3% and accuracy from 85.0 to 103.0% of the nominal value (Table 3).

Table 3: Precision and accuracy of Telmisartan in QC samples

Nominal Conc. (ng/mL)	Measured conc. (ng/mL)	Accuracy*	Precision (n=6)
12.0	10.8	90.0	5.3
	10.5	87.5	
	11.5	95.8	
	11.9	99.2	
	11.0	91.7	
	10.2	85.0	
900.0	848.3	94.3	3.6
	868.0	96.4	
	855.4	95.0	
	923.8	102.6	
	860.4	95.6	
	854.0	94.9	
1800.0	1840.3	102.2	4.7
	1817.1	101.0	
	1819.4	101.1	
	1630.0	90.6	
	1853.2	103.0	
	1816.3	100.9	

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

Recovery of QC Samples

The recovery of Telmisartan 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 4) for Telmisartan demonstrating the efficiency of analyte extraction in the presence of biological matrix.

Table 4: Recovery of Telmisartan in QC samples

QC sample	Number of Preparations	% Recovery
		Telmisartan
LQC	1	85.94
	2	91.21
	3	99.37
	4	101.37
	5	98.20
	6	89.94
MQC	1	86.95
	2	93.72
	3	96.67
	4	100.16
	5	95.41
	6	95.37
HQC	1	87.95
	2	94.26
	3	94.45
	4	96.16
	5	97.51
	6	98.66

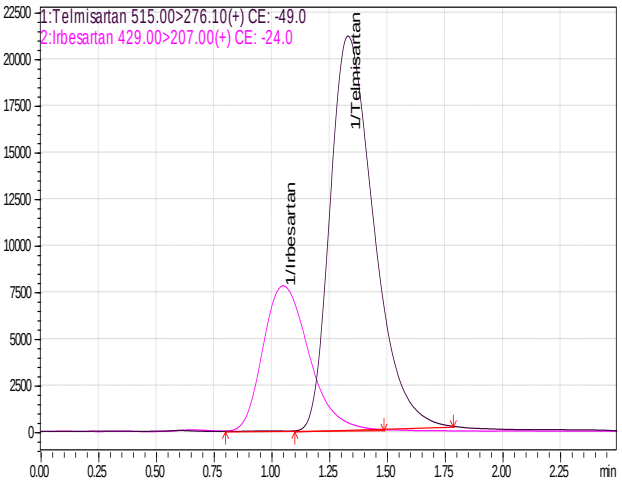

Figure 4 : Mass chromatogram of Telmisartan at MQC

❑ Conclusions

A simple, high throughput LC/MS/MS method for quantitative determination of Telmisartan in human plasma was developed. The LLOQ of the method was determined at 4.0 ng/mL. The linear dynamic range of the calibration curve was 4.0 – 2000 ng/mL with a regression of R^2 = 0.9970. Good recoveries (between 85.9 to 101.4 %) were obtained for all the three levels of QC samples repeatedly.

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