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Application News

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LCMS-8040 UFMS

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

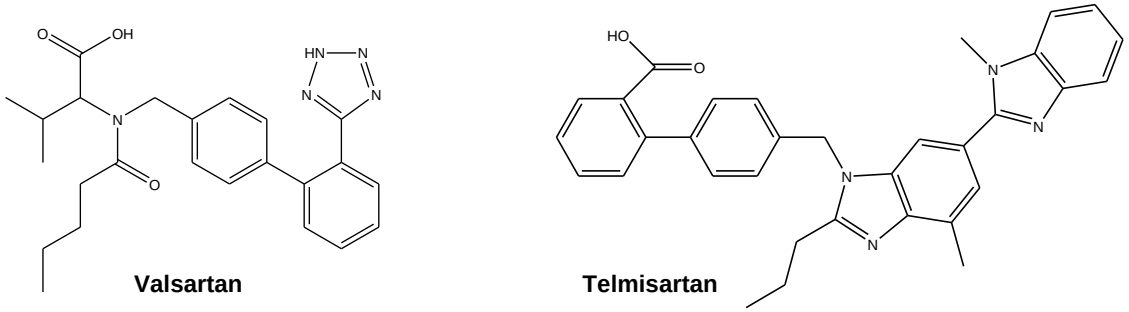
Manikandan Venkateswaran, Vinod Pillai. S.L, Venkat Manohar and Arvind Thyagarajan
*Indian Institute of Chromatography & Mass Spectrometry (IICMS)**

Abstract

A sensitive and fast LC/MS/MS method for the quantitative determination of valsartan in human plasma using UHPLC NEXERA coupled to LCMS-8040 triple quadrupole mass spectrometer was described. A simple protein precipitation method was employed to extract valsartan and the internal standard from the plasma samples.

❑ Introduction

Valsartan (N-Valeryl-N[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl valine) is an orally active, potent and specific competitive angiotensin II antagonist acting at the ATI receptor, which mediates all known effects of angiotensin II on the cardio vascular system. Valsartan is widely used in treatment of hypertension. Several analytical methods for determination of Valsartan have been reported in the literature. 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 Valsartan in human plasma with Telmisartan as internal standard is described using the Nexera UHPLC coupled to a LCMS-8040 instrument.



Valsartan **Telmisartan**

Figure1 Chemical structures of valsartan and Telmisartan (IS)

❑ Experimental

Preparation of aqueous standards: Stock solutions of Valsartan was prepared separately at 1mg/mL concentration in Water : Acetonitrile (20:80) mixture. The Internal standard (IS) stock solution of Telmisartan was prepared at 1mg/mL concentration in Methanol. The stock solution of internal standard was further diluted with Water : Acetonitrile (20:80) mixture to obtain an internal standard working solution (ISTD) at a concentration of 50µg/mL. The stock solution of Valsartan was then serially diluted with Water : Acetonitrile (20:80) mixture to obtain aqueous Valsartan standards at concentration 0.2, 0.5, 1, 2, 5, 7.5, 10, 20, 50, 75, 100, and 150 µg/mL respectively.

Preparation of plasma calibration standards (CC): 180µL of human plasma was spiked with 20 µL of each aqueous valsartan standard solution and vortexed for 30 seconds to obtain plasma calibration standard whose concentration ranged from 20 ng/mL to 15000 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): Three QC samples (LQC, MQC and HQC) were prepared in plasma at concentrations of 60, 6000 and 12000ng/mL respectively. Six individual preparations for every level was performed to evaluate precision and accuracy. Each of these samples were then extracted according to the procedure as described under sample preparation.

Sample preparation: A simple protein precipitation method was used to extract valsartan and internal standard from plasma matrix. All plasma samples were treated with 1000 µL of methanol and vortexed for 5 minutes. The samples were then centrifuged at 13,000 rpm for 10 minutes. The supernatant layer was directly taken for injection into the LC/MS/MS system.

The LC/MS/MS conditions are as summarized in Table 1. Precursor ions of valsartan and Telmisartan (IS) were determined by injecting a solution containing these

** Address: No. 7, Chakrapani Road, Guindy, Chennai - 600 032, Tamil Nadu, India*

Table 1: Analytical conditions

Column : Zorbax Eclipse Plus C18,
100 x 2.1mm, 1.8µm
Mobile phase-A : 0.2% v/v Formic acid in water
Mobile phase-B : Acetonitrile
Isocratic : A:B::60:40 v/v
Flow rate : 600 µL/min DL temp : 200 ^{0}C
Column temp : 50 ^{0}C Heat block: 200 ^{0}C
Drying gas : 15 L/min Interface : ESI
Nebulizing gas : 3.0 L/min Interface volt: 4.5 kV

For Valsartan
MRM : 436.00 → 291.00 Polarity : Positive
Dwell time : 100 ms CE : - 17.0V
Q1 pre-bias : -32.0V Q3 pre-bias : -30.0V

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

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compounds in the Q1 scan mode. Under these conditions, the analyte and the IS yielded predominantly the protonated molecular ions of m/z 436 and m/z 515 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 and all parameters were optimized. Based on this, the ion transitions of m/z 436.00 → 291.00 and m/z 515.00 → 276.10 were used as MRM for Valsartan and Telmisartan (IS) respectively.

❑ Results and Discussion

The concentration of valsartan at lower limit of quantification (LLOQ) was determined as 20.0 ng/mL. This was confirmed from the coefficient of variance (CV) being less than 20% for the six replicate injections of valsartan at this concentration. The overlay chromatograms at LLOQ concentration for the six replicate analysis are shown in Figure 2.

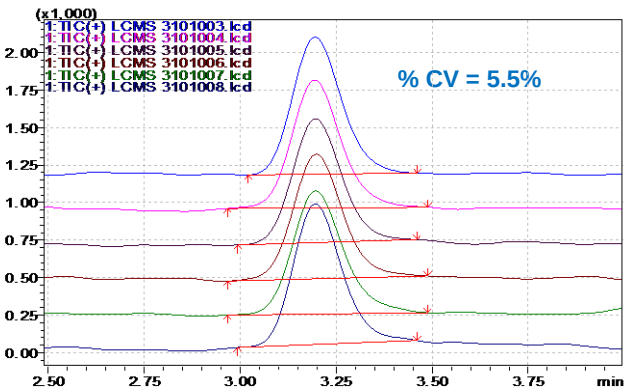

Figure 2: Overlay chromatograms of valsartan at LLOQ

The CC standards were used to construct a calibration curve by plotting the area ratio of Valsartan 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 20.0 to 15000.0 ng/mL was achieved for Valsartan with a R^2 value of 0.9997.

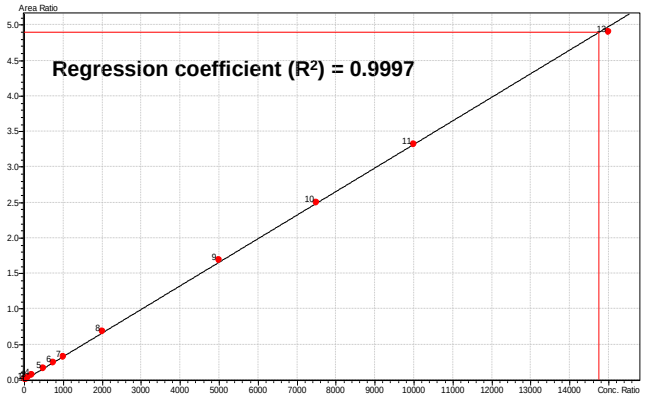

Figure-3 : Calibration curve of valsartan

Figure-3 shows a representative calibration curve of valsartan in plasma using irbesartan as internal standard. The calculated concentrations for all the CC standards were with in the ±10% of the nominal value as determined by bias calculation (refer Table 2).

Table 2 : Calculated concentrations of CC standards

Nominal Concentration (ng/mL)	Measured Concentration (ng/mL)	Accuracy*
20.0	19.9	99.7
50.0	50.1	100.2
100.0	101.6	101.6
200.0	199.6	99.8
500.0	495.2	99.0
750.0	731.5	97.5
1000.0	985.9	98.6
2000.0	2062.5	103.1
5000.0	5094.1	101.9
7500.0	7521.9	100.3
10000.0	9987.9	99.9
15000.0	14744.8	98.3

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

Low, middle and high QC samples containing valsartan was prepared at concentrations of 60, 6000 and 12000 ng/mL in plasma. The precision (%CV, n=6) for the QCs for Valsartan varied from 1.6 to 6.5% and accuracy from 81.7 to 101.5% of the nominal value (Table-3).

The recovery of Valsartan was calculated by comparing the peak area ratio 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 Valsartan demonstrating the efficiency of analyte extraction in the presence of biological matrix. The mass chromatogram of Valsartan at LQC and HQC levels are as shown in figure 4 and 5 respectively.

Table 3: Precision and accuracy of Valsartan in QC samples

Nominal Conc. (ng/mL)	Measured conc. (ng/mL)	Accuracy*	Precision (n=6)
60.0	58.2	97.0	3.9
	58.6	97.7	
	54.0	90.0	
	57.6	96.0	
	60.9	101.5	
	59.4	99.0	
6000.0	4902.6	81.7	6.5
	5389.5	89.8	
	5622.3	93.7	
	5043.7	84.1	
	5810.6	96.8	
	5550.7	92.5	
12000.0	10636.5	88.6	1.6
	10960.0	91.3	
	10761.8	89.7	
	10902.7	90.9	
	11146.9	92.9	
	10853.8	90.4	

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

Table 4: Recovery of Valsartan in QC samples

QC sample	Number of Preparations	% Recovery
		Valsartan
LQC	1	98.5
	2	99.2
	3	91.6
	4	97.6
	5	103.1
	6	100.6
MQC	1	96.9
	2	106.5
	3	111.1
	4	99.9
	5	114.8
	6	109.7
HQC	1	95.1
	2	98.0
	3	96.2
	4	97.5
	5	99.7
	6	97.0

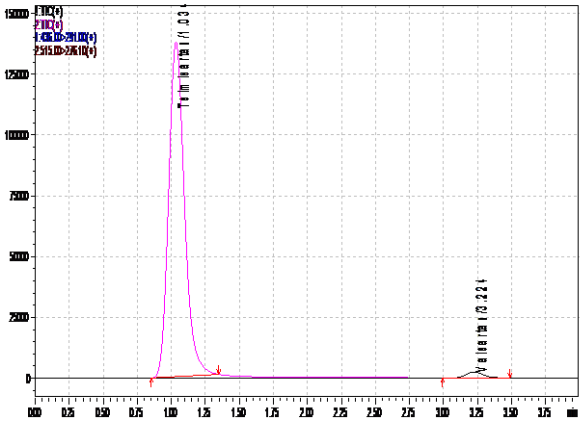

Figure 4: Mass chromatogram of valsartan at LQC

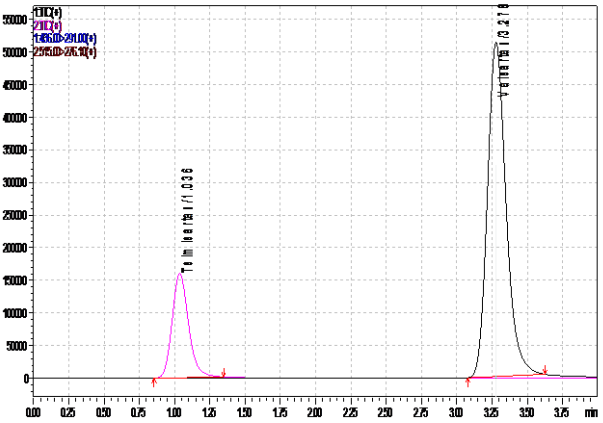

Figure 5: Mass chromatogram of valsartan at HQC

❑ Conclusions

A simple, high throughput LC/MS/MS method for quantitative determination of valsartan in human plasma was developed. The LLOQ of the method was determined as 20.0 ng/mL. The linear dynamic range of the calibration curve was 20.0 – 15000 ng/mL with a regression of R^2 = 0.9997. Good recoveries (between 91.6 to 114.8 %) were obtained at all the three levels of QC samples with repeatability.

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SHIMADZU (Asia Pacific) Pte. Ltd
79 Science Park Drive, #02-01/08 Cintech IV, Singapore 118264
www.shimadzu.com.sg
Tel: +65-6778 6280 Fax: +65-6778 2050