Original Article

ONE STEP QUANTIFICATION ANALYTICAL METHOD AND CHARACTERIZATION OF VALSARTAN BY LC-MS

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ABSTRACT

Objective: To develop precise LC-MS method for the estimation of valsartan impurities and perform validation as per ICH guidelines.

Methods: Valsartan (VLN) and its degradation products were analyzed by reverse phase high-performance liquid chromatography (RP-HPLC) using mobile phase water: acetonitrile: glacial acetic acid: phosphate buffer in the ratio of 500:500:1:0.5 v/v/v/v at 225 nm using column nucleosil C18, 125 ×4.0 mm, 5 µm. VLN sample (VLN SPL) thus obtained an unknown major impurity (UIMP) of 0.5 % at 0.38 retention time ratio (RRt) and purity of VLN was found to be 98.70 % respectively.

Results: Estimation of VLN SPL total unknown impurities was found to be 1.3% by RP-HPLC. In similarly by liquid chromatography mass spectroscopy (LC-MS) a typical chromatogram of valsartan (VLN) at Rt 9.03 min and UIMP at Rt 3.3 min were recorded at a total run time of 23 min. Assay of VLN SPL was validated as per international council for harmonization (ICH) guidelines. Average % recovery was found to be 100.04 % for VLN SPL.

Conclusion: The proposed work clearly indicates that the method can be easily adapted for the routine one step estimation of VLN active pharmaceutical ingredient (API).

Keywords: Valsartan, Acetonitrile, Methanol, LC-MS, Mobile Phase, Impurity

INTRODUCTION

Valsartan (VLN) [1-3] is a potent and highly selective oral drug that blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle and adrenal gland. It is chemicallyN-(1-oxopentyl)-N-[2¹-(1H-tetrazol-5-yl)-[1, 1-biphenyl]-4-yl] methyl]-L-valine. The guidelines recommended by ICH [4] state that the acceptable levels for a known and UIMP in an API should be less than 0.15 and 0.10 %, respectively. In the development of VLN, various processes of related IMP were observed. The reported literature includes an increasing number of publications, developments of analytical methods for analysis of tablets and biological samples by HPLC [5, 6], UPLC [7] and LC-MS [8, 9] methods. However, only few reports were found in the literature for the identification of IMP in VLN product. The previous established methods were found to have more Rt and total run time for analysis. The present study was aimed to develop for the identification and development of IMP by using RP-HPLC and LC-MS method in VLN sample. The structure of VLN and predicted UIMP structure was shown in fig. 1.
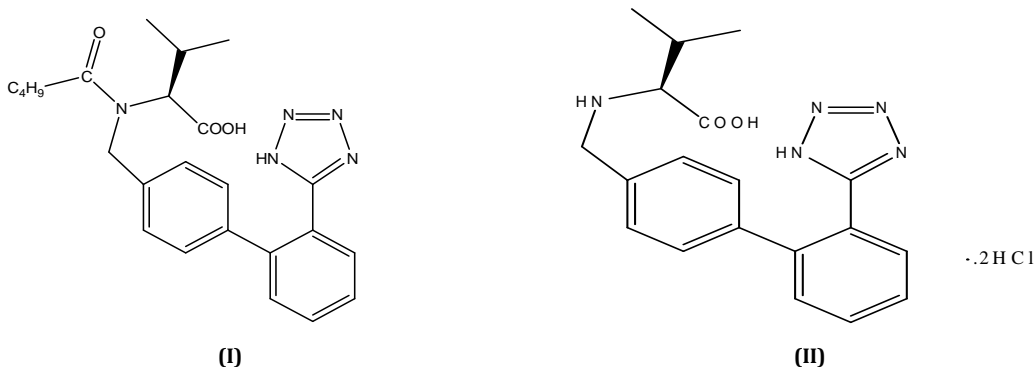

Fig. 1: Structure of valsartan (I) and unknown impurity (II)

MATERIALS AND METHODS

Chemical and reagents

Valsartan sample (VLN SPL) was obtained as a gift from Matrix (Mylan Pharmaceuticals) Lab Pvt Ltd, Hyderabad. glacial acetic acid, methanol, and acetonitrile are obtained from Merck chemicals, Mumbai.

Preparation of sample solutions

Accurately weighed about 50 mg of VLN SPL was transferred into a 100 ml volumetric flask, dissolve and make up to the mark with the mobile phase. Mobile phase mixture consists of water, acetonitrile, glacial acetic acid and phosphate buffer in the ratio of 500:500:1:0.5 v/v/v/v.

Instrumentation and chromatographic conditions

Shimadzu LC-20AD series HPLC and EZChrom Agilent 1100 HPLC were used. All weighing was done on Mettler toledo microbalance. LC-20AD series HPLC from Shimadzu technologies consists of an autosampler (Shimadzu SIL-HTC), LC-20AD serial pump, DGU-20A3 prominence degasser; analytical column nucleosil C18 HT (125 mm×4.0mm) 5 μm with a flow rate of 0.400 ml min-1. Data acquisition system and quantization program of applied biosystems analysis software version 1.5 was used for the determination of VLN and IMP. LC-MS analysis was performed on an API 5500 LC-MS triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface with turbo ion spray. The positive ions were measured in MRM mode (ion source 5500 v, declustering potential 57 v, focusing potential 256 v, entrance potential 10 v) for the analyte and IMP. The mass spectrometer was programmed to monitor the protonated molecule [M+H] for VLN at m/z 436 and for UIMP at m/z 352.

Assay procedure

Twenty tablets were weighed and crushed to fine powder. The tablet, powder equivalent to 50 mg of VLN working standard and 50 mg of processed VLN SPL was transferred into a 100 ml volumetric flask and diluted with the mobile phase separator. 10 ml of this solution was a pipette out and diluted to 100 ml with the mobile phase and sonicated for 5 min.

RESULTS AND DISCUSSION

VLN SPL of 500 μg ml-1 was analyzed by HPLC using mobile phase at 225 nm with a flow rate of 0.4 ml min-1. The percentage areas of related substances in the VLN SPL were recorded. The VLN SPL solution was injected into the LC-MS directly with the mobile phase and operates in positive ion mode. The chromatograms and spectrums were recorded. The previous reported HPLC [10-12] methods of VLN SPL and UIMP identification was found more Rt values when compared to proposed method, it shows that the proposed method was optimized and to separate more number of related IMP. A 10 μl of the VLN SPL solution was injected into the injector for six times under chromatographies condition. Area of each peak was measured at 225 nm. The amount of each drug present in the VLN SPL (n = 6) was determined from peak area and percent label claim and standard deviation (SD) was calculated as per ICH guidelines. The assay of observed results was shown in table 1 and fig. 2.

Table 1: Assay of valsartan API and % recovery

Drug	Label claim (mg/tablet)	Estimated % of labeled claim±SD	% Recovery±SD			
			80	100	120	Mean
VLN	5	150.51±0.286	100.08±0.543	99.986±0.432	100.03±0.632	100.365
VLN SPL	5	99.95±0.386	99.96±0.521	100.07±0.362	100.09±0.080	100.040

VLN: valsartan standard; VLN SPL: valsartan sample; SD: standard deviation, n=6
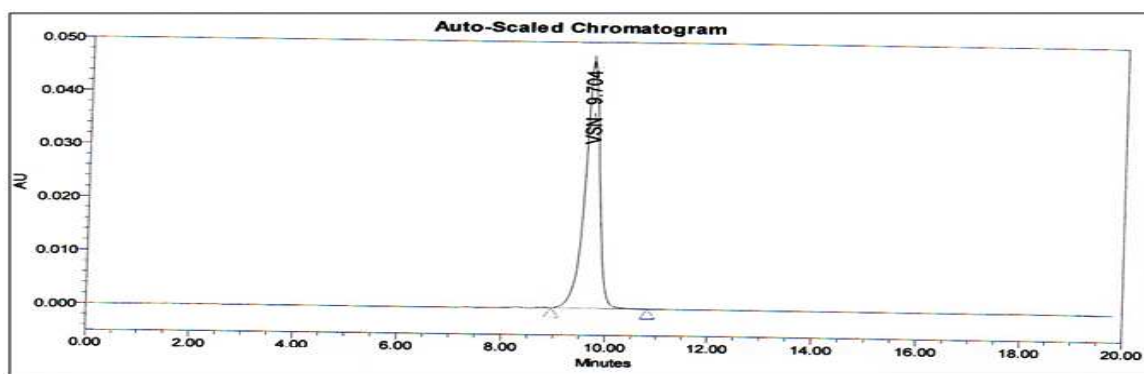

Fig. 2: Assay chromatogram of valsartan sample

A typical chromatogram of VLN SPL was analyzed and recorded by RP-HPLC and the purity of VLN was found to be 98.70 % and Rt 9.02 min, along with seven IMP peaks were identified at Rt of 3.46, 5.79, 6.73, 11.2, 12.5, 15.8 and 20.38 min respectively. In that chromatogram was identified major amount of UIMP [13] was observed at 0.5 % at Rt 3.46 and Rt ratio 0.38, which is higher than permissible limits of IMP limit. Total UIMP was found 1.3% as shown in fig. 3 and table 2. The VLN SPL was analyzed by LC-MS and the Rt of VLN and UIMP was found to be Rt 9.03 min and 3.461 min respectively, which are obtained from spectral data at 225 nm. The data and structures were shown in the fig. 4.

Table 2: RP-HPLC chromatogram for valsartan

Name	Rt	% Area	Rt Ratio
IMP	3.461	0.50	0.38
Peak 2	5.798	0.31	0.65
Rel. comp-B	6.734	0.28	0.74
VLN SPL	9.024	98.70	1.00
Peak 5	11.202	0.14	1.24
Peak 6	12.517	0.02	1.38
Peak 7	15.83	0.02	1.754
Peak8	20.38	0.03	2.25
Sum		100.0	

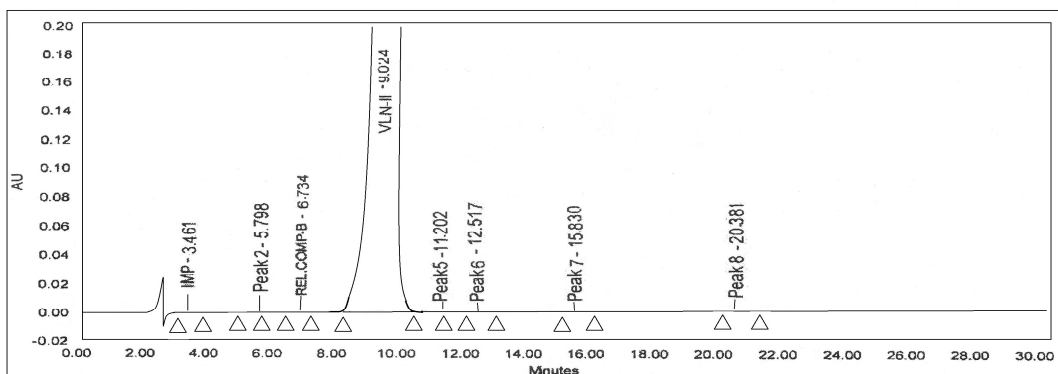Rt: retention time; VLN SPL: valsartan sample; IMP: impurity, n=6

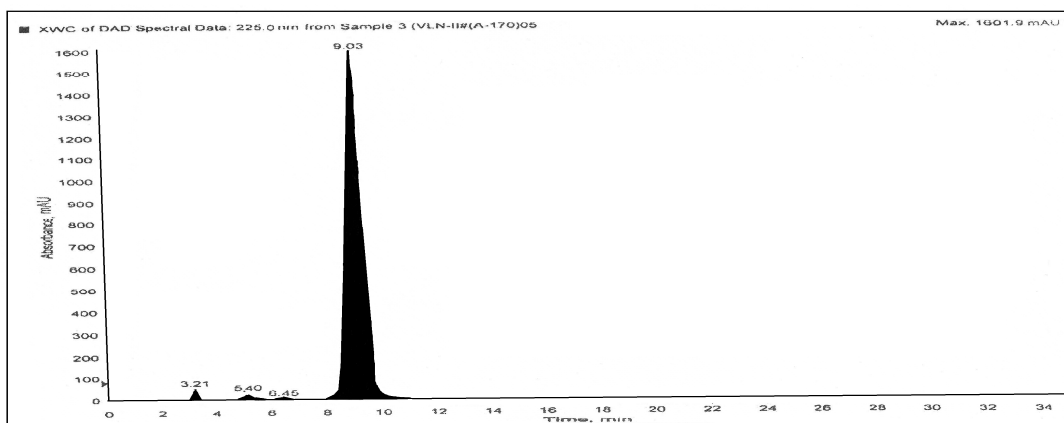**Fig. 3: A typical RP-HPLC chromatogram of VLN SPL**

Fig. 4: A typical LC-MS chromatogram of VLN SPL

From the analysis of spectral data of UIMP was determined by LC-MS method. From the above LC-MS Spectrum the m/z value of the IMP was suspected to be 352 (M+1) and XIC chromatogram was recorded at 352 amu and IMP eluted at Rt 3.46 min. The major IMP was formed in the VLN is due to the alkaline hydrolysis (KOH) of amide group in the process of VLN synthesis. The molecular ion is 83 amu less than that of the mass of VLN. Protonated molecular ion at m/z 352 (M+1) confirms the IMP monoisotopic mass value of 351 corresponding to the molecular formula $C_{19}H_{21}N_5O_2$ and molecular weight of valeryl group is 83. Therefore, the IMP was formed with the elimination of valeryl group from VLN and mass fragmentation was carried for molecular ion of UIMP. The mass fragments of the IMP were detected at m/z 306, 235 and 207 repectively, which were similar to that of the fragments obtained at Rt 3.461 min in LC-MS spectrum of the VLN and shown in table 3.

Table 3: MS data of valsartan sample

Name	LC-MS Rt (min)	Precursor ion (m/z)	Fragment ions (m/z)
VLN	9.0	436 (M+H)+	418,352,235,207
IMP	3.461	352 (M+H)+, 374 (M+Na)+, 390 (M+K)+	306,235, 207

VLN: valsartan; IMP: impurity; Rt: retention time

The predicted structure of UIMP of VLN may be is (S)-N-(1-carboxy-2-methylprop-1-yl)-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl] amine as shown in fig. 5. The assay of VLN SPL was developed and validated by RP-HPLC the results and % recovery was found to be 100.04. The method is precise within the acceptance limit of 2% and result was shown in table 4.

Table 4: Assay of valsartan

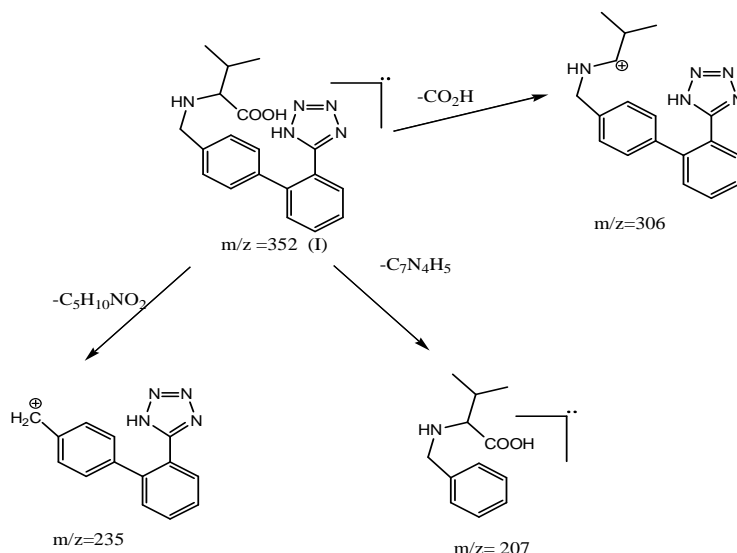| S. No.	Mean Area		Mean concentration		Mean moisture content		Standard potential mean	Assay mean
	Sample	Standard	Sample	Standard	Sample	Standard		
VLN	924478	900020	0.05021	0.05027	0.90	1.29	100	99.9

VLN: valsartan, n=6

Fig. 5: UIMP of VLN SPL fragmentation pattern structure

CONCLUSION

VLN SPL by RP-HPLC peak purity was identified as 98.70 %. The total related IMP were found 1.3 % by RP-HPLC. The intrinsic stability of VLN SPL and its related UIMP was identified by RP-HPLC and characterized by LC-MS. Proposed method development of VLN IMP was isolated. Total run time was 23 min and Rt of VLN API was 9.3 min, when compared with the earlier reported method, this method was fast and accurate. The study focussed on the development chromatographies condition support to identification of a number of related IMP from the process development of VLN synthesis. The characterized IMP structure, analytical data was compared by the fragmentation pathway of the API. As per USP acceptance criteria assay of VLN should not be less than 98.0 % and not more than 102.0 % w/w on anhydrous basis and assay value was found to be within the limits.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICTS OF INTERESTS

Declare none

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