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Full Length Original Research Paper

Validated reverse phase high performance liquid Chromatography method for simultaneous estimation of Valsartan Potassium and Amlodipine Besylate in tablet dosage form

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Abstract: This work is concerned with application of simple, economical, precise, accurate and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Valsartan potassium (VP) and Amlodipine besylate (AB) on RP C-18 Column (Inertsil ODS-2, 150 x 4.6 mm) using Methanol: Water (62:38), pH adjusted to 3.0 with O-phosphoric acid as mobile phase at a flow rate of 1.4 ml/min and the detection wavelength was 230 nm. The retention time for VP and AB was found to be 6.0 and 3.5 min, respectively. Proposed method was validated for precision, accuracy, linearity range, robustness and ruggedness.

Keywords: Valsartan potassium, Amlodipine besylate, Reverse Phase High Performance Liquid Chromatography, Inertsil, retention time, C-18 column.

Introduction

Amlodipine Besylate (AB) is chemically 3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridinedicarboxylate, mono-benzenesulphonate, is used for treating hypertension and angina pectoris [1] in the form of the besylate salt, Amlodipine besylate. It is official in IP [2], USP [3], EP [4] and BP [5] .Various analytical methods have been reported for the assay of Amlodipine besylate [6] in pure form as well as in pharmaceutical formulations. They include high performance liquid chromatography[7]- [12], reversed phase high performance liquid chromatography[13]- [16] , high performance thin layer chromatography [17]- [20], gas chromatography[21], gas chromatography–mass spectrometry[22], liquid chromatography with tandem mass spectrometry[23] and fluorimetry[24], derivative spectroscopy[25]- [26] simultaneous multicomponent mode of analysis and difference spectrophotometry

Valsartan is chemically (S)-N-(1-Oxopentyl)-N-[[2'-(1H-tetrazol-5-yl) [1,1'-biphenyl]-4-yl]methyl]-L-valine, is an orally active specific angiotensin II receptor blocker effective in lowering blood pressure in hypertensive patients[27].Various Analytical methods have been reported for the assay of Valsartan in pure form as well as in pharmaceutical formulations includes HPLC[28]-[30], LC-MS[31]- [33], Protein precipitation[34], Capillary electrophoresis[35] and simultaneous UV spectrophotometric methods[36]- [37].

Amlodipine and Valsartan keep blood vessels from narrowing, which lowers blood pressure and improves blood flow. The combination of Amlodipine and Valsartan is used to treat high

blood pressure (hypertension) [38]. This medication is usually given after others have been tried without successful treatment of hypertension [39] - [40].

Till now, no RP-HPLC study on simultaneous estimation of Amlodipine and Valsartan in tablet dosage form in pharmaceutical preparations has been found in literature survey. As no method is reported for Amlodipine and Valsartan in tablet formulation, the aim of the present study was to develop accurate, precise and selective reverse phase HPLC assay procedure for the analysis of Amlodipine and valsartan in its tablet dosage form.

Materials and Methods:

Chemicals and Reagents

Amlodipine Besylate and Valsartan are obtained as gift samples from Ranbaxy research Laboratories, Gurgaon and Hetero drugs, Hyderabad respectively. Methanol (HPLC grade), MilliQ water (HPLC grade), Potassium dihydrogen orthophosphate and orthophosphoric acid were of reagent grade. The Pharmaceutical preparation of combination of Amlodipine and Valsartan tablets that is going under product development phase at Ranbaxy research laboratories is available in the ratio of 10:160 mg as tablets.

Instrumentation

A Gradient HPLC system is used of Waters 2695 with PDA detector. The HPLC system was equipped with Empower software for data processing.

Selection of Chromatographic Condition

The mobile phase containing Methanol: water (68:32), pH adjusted to 3.0 with Orthophosphoric acid was found to resolve VP and AB. Orthophosphoric acid was used for pH adjustment of buffer. The mobile phase was filtered on a 0.45 micron membrane filter and then ultrasonicated for 30 min. The flow rate was set to 1.4mL min^{-1}. Both drugs showed good absorbance at 238 nm, which was selected as wavelength for further analysis. All determinations were performed at constant column temperature (15 ± 2°C).

In order to optimize the LC separation of AB and VP, initially, wavelength of 238nm was selected for the UV detection because at this wavelength there was maximum overlap of the spectra of Amlodipine and Valsartan. Nylon filter 0.45µm was selected for sample filtering as very good sample condition was observed against other filters. Retention of both the drugs was found dependent on pH of buffer. Amlodipine retention increases with increase in pH while Valsartan retention decreases with increase in pH. Both drugs were found sensitive to aqueous composition. A ten percent increase in aqueous composition resulted in 1.6 and 1.8 times increase in retention for amlodipine and Valsartan respectively. The buffer solution of pH3.0 and mobile phase composition of Methanol: water (62:38) was found most appropriate for separation of amlodipine and valsartan on Inertsil ODS-2 (150*4.6) mm, 5µm column. Flow rate of 1.4mL min^{-1} selected based on capacity factor and column efficiency. Amlodipine and Valsartan were well resolved in reasonable time of 8 minutes. The retention times were 3.5 min and 6.0 min, respectively. The resolution between amlodipine and valsartan was 14. The interference with the blank was within the limit of 1 percent. The peak purity of the peaks of Amlodipine and Valsartan was tested using PDA detector and were found to be pure.

Preparation of Stock Solutions

Standard stock solutions containing Valsartan Potassium (VP) and Amlodipine besylate (AB) were prepared by dissolving 25mg equivalent weight of Amlodipine and 160mg of Valsartan to a 250mL and 100mL volumetric flask respectively. 50mL of Methanol was added to each flask and sonicated for 20min to dissolve. Volume was made up to mark with mobile phase. 10mL of each solution was transferred to 100mL volumetric flask and the volume was made with mobile phase. Combined solution was filtered through 0.45 µm nylon filters to get stock solutions containing 160µgmL^{-1} of VP and 10µgmL^{-1} of AB respectively.

Sample Preparation

20 intact tablets were transferred to a 1000mL volumetric flask to which 100mL of Methanol was added and kept in ultrasonic bath for 10 minutes for complete dispersion of tablets. The volume was made up to mark with mobile phase. 5mL of the solution was transferred to 100mL volumetric flask and the volume was made with mobile phase and filtered through 0.45 µm nylon filter. The diluted solution was analyzed under optimized chromatographic conditions and chromatogram is depicted in figure no.1.

Method Validation [41]

The proposed HPLC method was validated as per ICH guidelines.

Specificity

The peak purity of VP and AB were assessed by comparing the retention time (TR) of standard VP and AB. Good correlation was obtained between the retention time of standard and sample of VP and AB. Peak purity is described in figure no. 2 and figure no. 3 respectively for amlodipine and valsartan peaks. Results are shown in Table 1.

Linearity and Range

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for VP and AB were found to be 112µgmL^{-1} to 208µgmL^{-1} and 07µgmL^{-1} to 13µgmL^{-1} respectively. The regression equation for VP and AB were found to be y = 28.30x + 63.62 and y = 28.78x + 98.30 with coefficient of correlation, (r) 0.99936 and 0.99914, respectively. Results are shown in Table 1.

Precision

Repeatability

Repeatability was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise. Results are shown in Table 1.

Intermediate precision

Six independent sample solutions were prepared as per methodology and Intermediate precision was performed in duplicate on different HPLC system with different column on different date by different analyst but on same homogeneous sample batch. Overall %RSD was found to be less than 2%. Results are shown in Table 1.

Accuracy (Recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard VP and AB were added to pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies are shown in Table 1.

Robustness of method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, pH, organic, temperature and wavelength ratio on the retention time and tailing factor were studied. The method was found to be unaffected by small changes like ± 0.2 change in pH, ± 10% change in flow rate and ± 2% change in organic, ± 5°C change in temperature, ± 5nm change in wavelength. Results of robustness studies are shown in Table 2.

Results and Discussion

To develop a precise, accurate and suitable RP-HPLC method for the simultaneous estimation of VP and AB, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. The results obtained by the validation of the combined formulation are summarized in Table 1. System suitability tests were carried out as per ICH guidelines are summarized in Table.2.

Conclusions

The proposed method is simple, sensitive and reproducible and hence the method can be used in routine for simultaneous determination of VP and AB in bulk as well as in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The RSD for all parameters was found to be less than one, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of VP and AB in multi component pharmaceutical preparation.

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Table 1: Method Validation Parameters

Parameters	Valsartan Potassium	Amlodipine Besylate
Specificity (% difference)	0.2%	0.7%
Linearity (range)	112-208 µg/ml	07-13 µg/ml
Linearity (correlation coefficient)	0.99936	0.99914
Repeatability (% RSD*)	0.20	0.37
Intermediate Precision (%RSD*)	1.07	1.23
Accuracy (% recovery)	100.93	100.01
Accuracy (%RSD*)	1.08	0.82

*Relative Standard Deviation

Table 2: System Suitability Parameters and Robustness

Component	Robustness parameter	k'[a]	T[b]	R[c]	N[d]	%Assay
Amlodipine Besylate	No change (repeatability)	4.7	0.8	14	4270	100.02
	Flow (+0.2 mL min^{-1})	4.3	1.4	11	3673	107.6
	Flow (-0.2 mL min^{-1})	4.2	1.2	13	3947	108.3
	Column Temperature(40°C)	4.6	0.9	14	3229	100.08
	Column Temperature(30°C)	4.6	1.1	12	3053	100.04
	Wavelength(237nm)	4.9	1.3	12	4126	100.97
	Wavelength(227nm)	4.4	1.4	10	4059	101.32
	pH of buffer(3.2)	5.3	1.7	16	3613	101.34
	pH of buffer(2.8)	4.8	1.5	14	3532	101.18
Valsartan potassium	No change (repeatability)	1.7	0.8	14	5042	100.02
	Flow (+0.2 mL min^{-1})	1.5	1.2		4674	98.6
	Flow (-0.2 mL min^{-1})	1.9	1.7		4988	98.3
	Column Temperature(40°C)	1.4	1.1		4952	99.2
	Column Temperature(30°C)	1.7	1.1		4886	97.4
	Wavelength(237nm)	1.3	0.9		4860	98.3
	Wavelength(227nm)	1.4	1.1		4598	98.4
	pH of buffer(3.2)	1.9	1.3		3742	97.8
	pH of buffer(2.8)	1.8	1.5		4015	96.6

[a]Capacity factor determined for individual peak. [b]Tailing factor determined for individual peak. [c]Resolution factor determined for peaks between amlodipine and valsartan peaks. [d]Number of theoretical peaks for individual peaks.

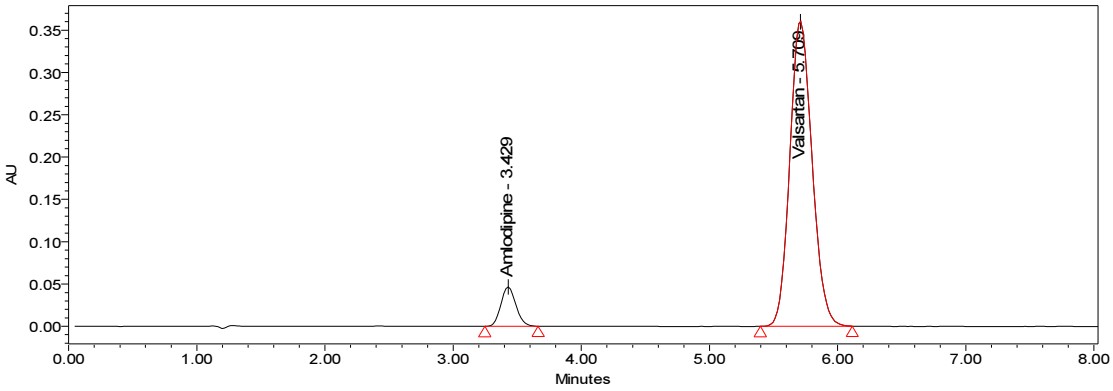

Figure 1: Chromatogram for sample injected for amlodipine and valsartan

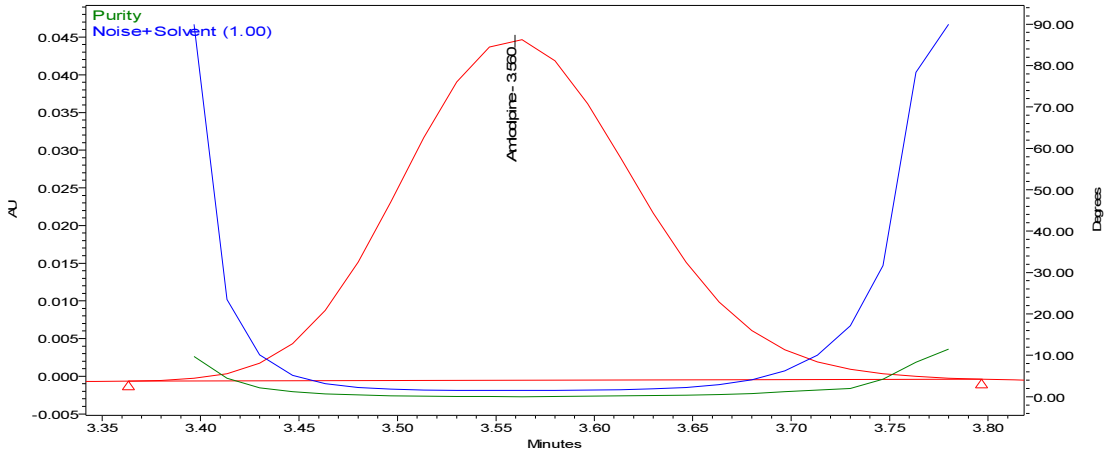

Figure 2: Peak purity plot of amlodipine besylate

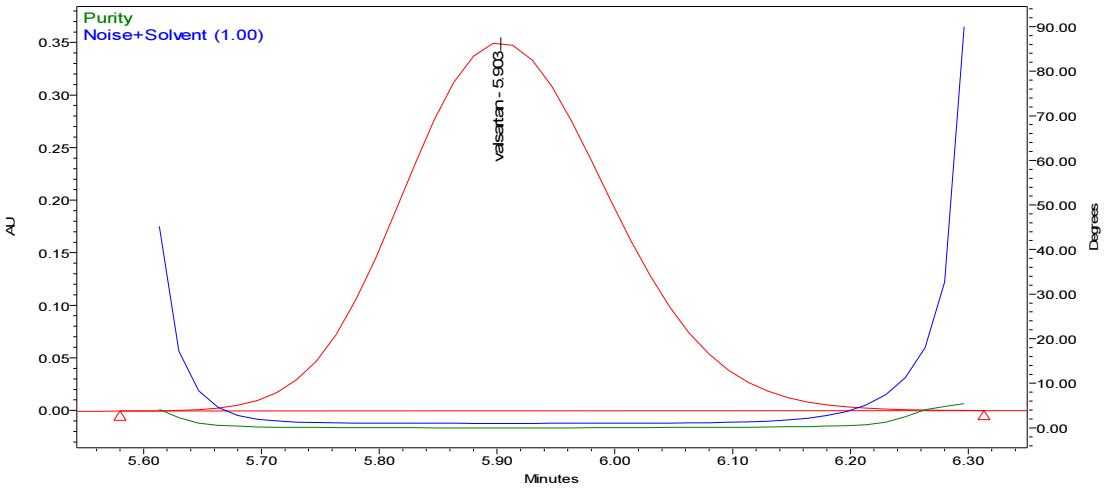

Figure 3: Peak purity plot for valsartan potassium

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