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A STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF VALSARTAN AND ATORVASTATIN FROM THEIR COMBINATION DRUG PRODUCT

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ABSTRACT

A simple, precise and accurate stability indicating RP-HPLC method has been developed and subsequently validated for simultaneous estimation of Valsartan and Atorvastatin from their combination dosage form. Water's HPLC equipped with UV-Visible and Diode Array detectors, with Empower software was used. Column used was Hypersil BDS C18, 5 µm, 250 mm × 4.6 mm i.d., at 40°C. Mobile phase consisting of a mixture of 0.1% acetic acid and acetonitrile in the ratio 50:50 v/v respectively at a flow rate of 2.0 mL/ min and UV detection was carried out at 225 nm for Valsartan and 246 nm for Atorvastatin. Valsartan and Atorvastatin in their combined dosage form were exposed to thermal, photolytic, oxidative, acid-base hydrolytic stress conditions, the stressed samples were analyzed by proposed method. Peak purity results suggested no other co-eluting, interfering peaks from excipients, impurities, or degradation products due to variable stress condition, and the method is specific for the estimation of Valsartan and Atorvastatin in presence of their degradation products and impurities within 7 minutes. The retention time of Valsartan and Atorvastatin were 3.33 and 5.44 minutes respectively. The method was found linear over the range of 5-15 µg per mL for Atorvastatin and 40-120 µg per mL for Valsartan. The proposed method was validated as per the ICH and USP guidelines.

Key words: Valsartan, Atorvastatin, RP-HPLC method.

INTRODUCTION

Atorvastatin

Atorvastatin calcium(Fig.1) is [R-(R*, R*)]-2-(4-fluorophenyl)-β, δ-dihydroxy-5-(1-methyl ethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1Hpyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate. The empirical formula of atorvastatin calcium is $(C_{33}H_{34}\ FN_2O_5)\ 2Ca{\bullet}3H_2O$ [1] and its molecular weight is 1209.42.

Atorvastatin calcium is a white to off-white crystalline powder that is insoluble in aqueous solutions of pH 4 and below. Atorvastatin calcium is very slightly soluble in distilled water, pH 7.4 phosphate buffer, and acetonitrile; slightly soluble in ethanol; and freely soluble in methanol.

Valsartan (VAL) (Fig.2) chemically, N-(1-oxopentyl)-N-[(2'-(1H-tetrazol-5-yl)(1,1'-biphenyl)-4 -yl) methyl]-L-valine, is a potent angiotensin receptor blocker.

Stability testing is an important part of the process of drug product development [2]. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors, such as temperature, humidity, and light, and enables recommendation of storage conditions, retest periods, and shelf lives to be established. The two main aspects of a drug product that play an important role in shelf life

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determination are assay of the active drug and degradation products generated during the stability study. The drug product in a stability test sample needs to be determined using a stability indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines and U.S. Pharmacopia (USP)[2-5] . Although stability indicating methods have been reported for assay of various drugs in drug products, most of them describe assay procedures for drug products containing only one active drug substance. Only few stability indicating methods are reported for assay of combination drug products containing two or more active drug substances [6-10]. The objective of this work was to develop a simple, precise, and rapid column liquid chromatography (LC) procedure that would serve as stability indicating assay method for combination drug product of Valsartan and Atorvastatin.

Fig. 1: Chemical structure of Atorvastatin.

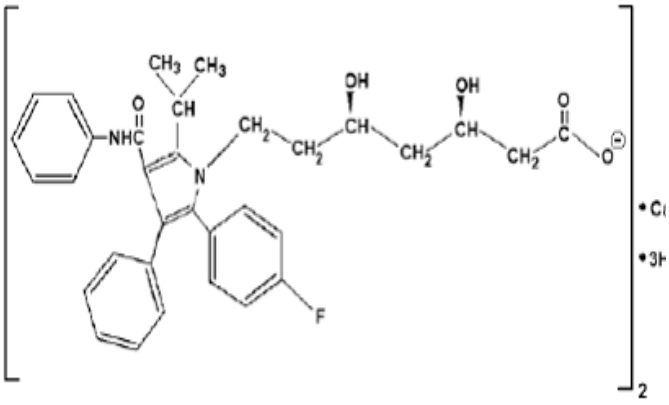
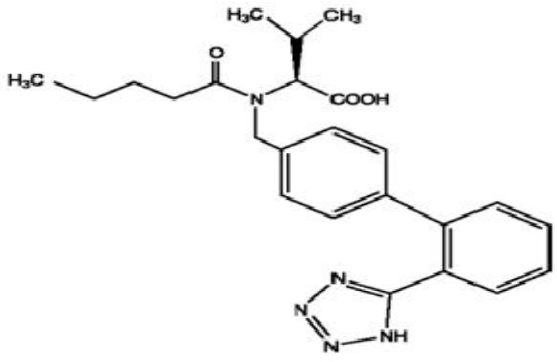


Fig. 2: Chemical structure of Valsartan.



MATERIALS AND METHODS

Instrumentation

The present work was carried out on Water's HPLC equipped with UV-Visible and Diode Array detectors with pair of 10 mm matched quartz cells. Glassware's used were of 'A' grade and were soaked overnight in a mixture of chromic acid and sulphuric acid, rinsed thoroughly with double distilled water and dried in hot air oven [11-14].

Reagent and chemicals

Milli-Q water: - (MILLIPORE SAS 67120, France), HPLC grade Acetonitrile (RANKEM, India), Acetic acid and Hydrochloric acid ("RANKEM", RFCL Ltd, New Delhi), LR grade 30 %, Hydrogen Peroxide and Sodium Hydroxide (MERCK, India) were used.

Instrument and conditions

Chromatography was performed with Water's HPLC equipped with UV-Visible & Diode Array detectors. The LC separations were performed at 40°C on an Hypersil BDS C18, 5 µm, 250 mm × 4.6 mm chromatographic column and Empower software was used for LC peak integration. The mobile phase was degassed by sonication with an Ultrasonic bath (Transonic Digital s, ELMA). The standard substances were weighed on Analytical balance (Mettler Toledo, AG285, Switzerland), stability studies were carried out in a Photo stability chamber (SVI equipments, Germany). Mobile phase consisting of 0.1% Acetic acid and acetonitrile in the ratio of 50:50v/v at a flow rate of 2.0 mL/min and UV detection was carried out at 225 nm for Valsartan and 246 nm for Atorvastatin with injection volume of 10 µL and chromatographic run time of 7 minutes.

Diluent-1 was a mixture of Acetonitrile and Methanol in the ratio of 50:50v/v respectively.

Diluent-2 was a mixture of Water and Methanol in the ratio of 50:50v/v respectively.

Preparation of Valsartan and Atorvastatin Standard Stock Solutions

Standard stock solutions of Valsartan (800 µg/mL) and Atorvastatin (250 µg/mL) were prepared separately in diluent-1. From this, a working concentration of 80 µg/mL for Valsartan and 10 µg/mL for Atorvastatin was obtained by transferring 5 mL of Valsartan standard stock solution and 2 mL of Atorvastatin standard stock solution in to 50 mL volumetric flask and diluted to volume with diluent-2 [15-19].

Analysis of the Marketed Formulation

Ten tablets were weighed and transferred into a 250 mL volumetric flask. About 25 mL of Milli-Q water was added and sonicated to disintegrate the tablets completely. 150 mL of diluent-1 added, solution was sonicated for 30 minutes with intermediate shaking and diluted to 250 mL with diluent-1 and mixed. The resulted mixture was centrifuged at 4000 RPM for 10 minutes; 5mL of the clear solution was transferred to 200 mL volumetric flask and made up to volume with diluent-2. After dilution 10 µL solution was injected for chromatographic analysis [19-22].

RESULTS AND DISCUSSION

Method Development

Literature survey revealed number of reported methods for Valsartan and Atorvastatin alone and with combination of other drug, but no stability indicating method was reported for Valsartan and Atorvastatin in combine dosage form. To develop accurate, precise and specific stability indicating RP-HPLC method for simultaneous estimation of Valsartan and Atorvastatin using stressed samples various mobile phase with different composition and flow rate were tried. After number of trial experiments, it was established that based on chemistry and pKa values of Valsartan and Atorvastatin, mobile phase buffer was chosen to be 0.1% acetic acid and organic modifier Acetonitrile. Buffer and organic phase (AcN) ratio 50:50 v/v optimized for better separation at a flow rate of 2.0 mL/min. UV detection at 225 nm for Valsartan and 246 nm for Atorvastatin with injection volume of 10 µL and column temperature 40 °C were found to be best for analysis.

Forced Degradation Studies of Drug Product

Chemical stress study

Acidic conditions

Ten tablets were transferred into a 250mL round bottom flask, 25mL of Milli-Q water added and sonicated till the tablets disintegrated completely. Then 10mL of 0.5M HCl was added and the solution was refluxed at 60°C for 3 hours. Cooled to room temperature, neutralized with 0.5M NaOH solution, added 150mL of diluent-1 and sonicated for 30 minutes with intermediate shaking. Total volume was made up to 250mL with dilent-1. 10mL of the sample was centrifuged at 4000 RPM for 10 minutes; 5mL of the clear solution was transferred to 200 mL volumetric flask and made up to volume with diluent-2.

Placebo sample was also subjected to the above stress condition and injected into the HPLC.

Alkali conditions

Ten tablets were transferred into a 250mL round bottom flask, 25mL of Milli-Q water added and sonicated till the tablets disintegrated completely. Then 10mL of 0.5M NaOH was added and the solution was refluxed at 60°C for 3 hours. Cooled to room temperature, neutralized with 0.5M HCl solution, added 150mL of diluent-1 and sonicated for 30 minutes with intermediate shaking. Total volume was made up to 250mL with dilent-1. 10mL of the sample was centrifuged at 4000 RPM for 10 minutes; 5mL of the clear solution was transferred to 200 mL volumetric flask and made up to volume with diluent-2.Placebo sample was also subjected to the above stress condition and injected into the HPLC.

Oxidation conditions

Ten tablets were transferred into a 250mL round bottom flask, 25mL of Milli-Q water added and sonicated till the tablets disintegrated completely. Then 10mL of 10% hydrogen peroxide was added and the solution was kept on a rotary shaker at room temperature for 12 hours, added 150mL of diluent-1 and sonicated for 30 minutes with intermediate shaking. Total volume was made up to 250mL with dilent-1. 10mL of the sample was centrifuged at 4000 RPM for 10 minutes; 5mL of the clear solution was transferred to 200 mL volumetric flask and made up to volume with diluent-2.

Placebo sample was also subjected to the above stress condition and injected into the HPLC.

Physical stress study

Drug product and placebo were exposed to 1.2 million lux hours of visible light in a light chamber and analyzed.

Drug product and placebo were exposed to 200 Watt Hours/m^2 of UV light in a light chamber and analyzed.

Drug product and placebo were stressed at 105°C for 24 hours and analyzed.

Drug product and placebo were stressed at 90% RH at 25°C for 7 days and analyzed.

No significant degradation was observed in the physical stress condition. The % degradation in stress condition for samples with peak purity results were given in Table 1.

Method Validation

The described method has been validated, in addition to its specificity, for linearity, system suitability, accuracy, and intermediate precision. The standard solutions for linearity were prepared at different concentration levels. Characteristic parameters for the regression equation and system suitability are given in Table 2.

Repeatability of measurements of assay of the tablets was evaluated using six replicate preparations. The intra- and inter-day variation for the determination of Valsartan and Atorvastatin were evaluated and found coefficient of variation (CV) values of within-day and day-to-day variations revealed that the proposed method is precise (Table 3). Accuracy of method was checked by a recovery study using the placebo and drug substance at 3 different concentration levels in triplicate. Results of the recovery study are shown in Table 4. The method was found to be robust with small variations in flow rate (±0.2 mL/min) and concentration of acetontrile (±10%) in the mobile phase as there was no significant difference in peak area and retention time.

Table 1. Results of forced degradation study using the proposed method

Stress condition stress	% Net degradation	Valsartan			Atorvastatin		
		Purity angle	Purity threshold	Purity flag	Purity angle	Purity threshold	Purity flag
Stressed with 0.5M HCl for 3 hrs at 60°C		0.024	0.326	NO	0.056	0.322	NO
Stressed with 0.5M NaOH for 3 hrs at 60°C		0.030	0.423	NO	0.080	0.426	NO
Stressed with 10% H2O2 for 3 hrs at 60°C		0.022	0.288	NO	0.044	0.634	NO
Exposed to 1.2 million lux hours of visible light		0.046	0.326	NO	0.055	0.288	NO
Exposed to 200 Watt Hours/m^2 of UV light		0.028	0.434	NO	0.032	0.340	NO
Heated at 105°C for 24 hours		0.033	0.500	NO	0.028	0.298	NO
Exposed to 90% RH at 25°C for 7 days		0.036	0.438	NO	0.040	0.310	NO

Table 2. System suitability parameter of proposed RP-HPLC method

Parameter	Observed value	
	Valsartan	Atorvastatin
Retention time	3.33	5.44
Tailing factor	1.0	1.1
% RSD of 5 replicate injection of standard	0.3	0.5

Table3. Intra and Inter-day precision data of proposed RP-HPLC method

Intra-day precision

S.No	Valsartan	Atorvastatin
1	98.2	100.6
2	98.9	99.6
3	99.3	100.4
4	98.9	98.9
5	99.1	99.1
6	98.7	99.8
Aveage	98.9	99.7
% RSD	0.4	0.7

Inter-day precision

S.No	Valsartan	Atorvastatin
1	98.6	100.2
2	99.6	100.2
3	99.7	99.5
4	98.4	99.2
5	98.7	100.4
6	98.8	100.5
Aveage	99.1	99.9
%RSD	0.6	0.5

Table 4. Recovery data for proposed RP-HPLC method (n=3)
For Valsartan

Spike level	'µg/mL' added	'µg/mL' recovered	Mean % Recovery
50%	399.7	396	99.1
100%	800.2	794.1	99.2
150%	1200.5	1186.9	98.9

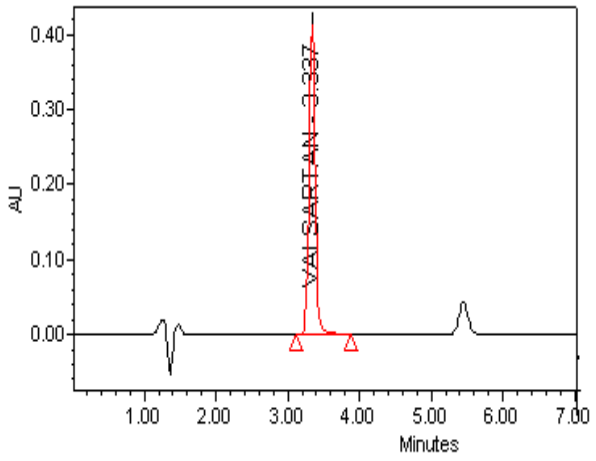
n=Number of determinations

Table 4. Recovery data for proposed RP-HPLC method (n=3) contd.,
For Atorvastatin

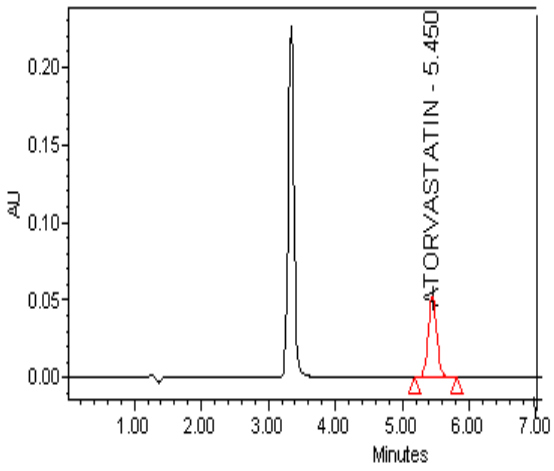
Spike level	'µg/mL' added	'µg/mL' recovered	Mean %Recovery
50%	49.9	49.6	99.4
100%	100.3	99.9	99.6
150%	150.2	150	99.9

n=Number of determinations

Typical chromatogram for valsartan at 225 nm:



Typical chromatogram for Atorvastatin at 246 nm:



CONCLUSION

Based on peak purity results, obtained from the analysis of forced degradation samples using described method, it can be concluded that there is no other co-eluting peak with the main peaks and the method is specific for the estimation of Valsartan and Atorvastatin in presence of their degradation products. The method has linear response in stated range and is accurate and precise. Though no attempt was made to identify the degradation products, described method can be used as stability indicating method for assay of Valsartan and Atorvastatin in their combined dosage form.

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