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Research Article

A STABILITY INDICATING RP-HPLC METHOD WITH SHORTER RUNTIME FOR SIMULTANEOUS ESTIMATION OF VALSARTAN AND AMLODIPINE 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 (VAL) and Amlodipine (AML) from their combination dosage form. Water's HPLC equipped with UV-Visible and Diode Array detectors, with Empower software was used. Column used was XTerra® RP8, 5 µm, 100 mm × 4.6 mm i.d., at 30° C.Mobile phase consisting of 0.05M Ammonium Acetate and 0.5% TEA buffer having pH 5.5and Acetonitrile in the ratio of 68:32 v/v at a flow rate of 1.0 mL/ min and UV detection was carried out at 238 nm and 271 nm for AML and VAL, respectively. VAL, AML and 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 VAL and AML in presence of their degradation products and impurities within 6 minutes. The retention time of Valsartan and Amlodipine were 1.98 and 4.03 minutes respectively. The method was found linear over the range of 1-20 µg per ml for Amlodipine and 1.6-240 µg per ml for Valsartan. The proposed method was validated as per the ICH and USP guidelines.

INTRODUCTION

Amlodipine besylate (AML), 2 - [(2 - amino ethoxy) - methyl] - 4 - (2 – chloro phenyl) -1, 4 -dihydro - 6 - methyl - 3, 5 - pyridine dicarboxylic acid 3 - ethyl - 5 - methyl ester, benzene sulfonate (Fig. 1), is a potent dihydro calcium channel blocker.

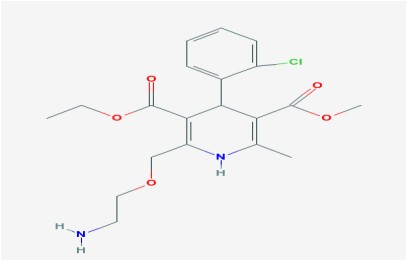


Fig. 1: Chemical structure of Amlodipine besylate

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

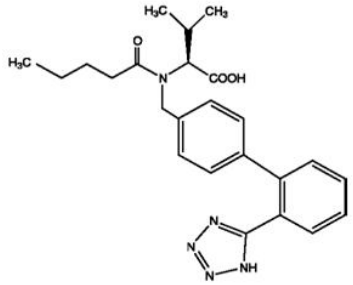


Fig. 2: Chemical structure of Valsartan

The combination of VAL and AML has been shown to be effective in the management of hypertension. The combination was generally more effective than individual drug therapy.

Stability testing is an important part of the process of drug product development. 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 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)[1-9] . 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. 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 VAL and AML.

VAL is official in USP, while AML is official in IP,BP, EP and USP in which HPLC method is describe for both drug in alone. The combination of VAL and AML is not official in any pharmacopoeia. Literature survey revealed HPLC, RP-HPLC, HPTLC, LCMS/MS, LC-MS and simultaneous UV Spectrophotometric methods are reported for the estimation of AML alone or in combination with other anti-hypertensive agents. Methods such as HPLC, LC-MS, Protein precipitation, Capillary electrophoresis and simultaneous UV spectrophotometer methods are reported for estimation of VAL alone or in combination with other agents. Stability indicating RP-HPLC assay method for AML alone and in combination with atorvastatine calcium and benazepril hydrochloride[10-32], respectively are available in literatures but no effective shorter Run time methods were reported for AML and VAL in combination. The aim of the present study was to develop accurate, precise and selective reverse phase HPLC assay procedure for the analysis of AML and VAL in bulk drug samples and in combined dosage formulation.

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.

Reagent and chemicals

VAL and AML Working standards were kindly gifted by Lupin Pharma, pune, India, with purity of 98.50% and 99.87%, respectively and were used without further purification for the study. The commercial fixed dose combination product containing 160 mg VAL and 10 mg AML was procured from Torrent Research Centre. Milli-Q water: - (MILLIPORE SAS 67120, France), HPLC grade Acetonitrile and Methanol (RANKEM, India), AR grades TriEthyl Amine (Qualigens fine chemicals, Mumbai), Ortho phosphoric Acid and Ammonium Acetate ("MERCK" Specialties Pvt Ltd, Mumbai), Hydrochloric acid ("RANKEM", RFCL Ltd, New Delhi) and 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 30°C on an XTerra® RP8, 5 µm, 100 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 0.05M Ammonium Acetate and 0.5% Tri Ethyl Amine(TEA) buffer having pH 5.5 and Acetonitrile in the ratio of

68:32v/v at a flow rate of 1.0 mL/min and UV detection was carried out at 238 nm and 271nm for AML and VAL, respectively with injection volume of 20 µL and Chromatographic Run Time of 6minutes.
Diluent was used mixed pH5.5 Acetate buffer and Methanol in the ratio of 50:50v/v respectively.

Preparation of VAL and AML Standard Stock Solutions

Standard stock solutions of VAL (1600 µg/mL) and AML (220 µg/mL) were prepared separately in diluent. For calibration curve series of mixed working standard solutions were prepared by transferring 0.1, 1, 3, 5, 8, 10, 12 and 15 mL of VAL standard stock solutions and 0.45, 0.75,1,2,3,5, 7, 9.5 mL of AML aliquots of standard stock solutions in to 100 mL volumetric flask and diluted to mark with diluent.

Analysis of the Marketed Formulation

Twenty tablets were accurately weighed, their Average weight was determined, and were ground to fine powder in a glass mortar. An amount of the powder equivalent to 10 tablets was dissolved in 175 mL of Methanol, solution was sonicated for 30 minutes with intermediate shaking and diluted to 250 mL with Methanol and mixed. The resulted mixture was centrifuged at 4000 RPM for 10 minutes; first 5 mL of the filtrate was discarded. From the filtrate pipetted 5 mL of aliquot was transferred to 200 mL volumetric flask and made up to volume with Diluent. After dilution 20 µL solution was injected for chromatographic analysis.

Forced Degradation Studies of Drug Product

An amount of the tablet powder equivalent to 12 tablets was taken in the series of six different round bottom flasks. In each of the flask 15 mL of Mehanol was added to dissolve active contents. Then after above six flasks were grouped in three sets each of two.
In two flasks of set I, 10 mL each of 0.5M and 0.1M HCl were added separately for acid degradation of VAL and AML, respectively.
For base hydrolysis, in two flasks of set II, 10 mL each of 0.5M and 0.1M NaOH were added separately for VAL and AML, respectively.
Finally, in two flasks of set III, 10 mL each of 30% and 10% H2O2 were added separately for VAL and AML, respectively.
After that above acidic, basic and oxidized solutions of VAL were refluxed for 2 h, 12 h and 45 min, respectively and solution of AML for 1 h, 20 min and 15 min, respectively.
The tablet powder were also exposed to thermal stress at 105°C in oven for 72 h and photo stability chamber at 1.2 million lux hours.
After sufficient degradation under acid/base hydrolysis, oxidative, thermal and photo degradation stress condition tablet contents were dissolved and diluted to achieve solutions having final concentration 160 mg/mL for VAL and 10 mg/mL for AML.

RESULTS AND DISCUSSION

Method Development

Literature survey revealed number of reported methods for VAL and AML alone and with combination of other drug, but no stability indicating method was reported for VAL and AML in combine dosage form. To develop accurate, precise and specific stability indicating RP-HPLC method for simultaneous estimation of VAL and AML 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 Amlodipine mobile phase buffer pH chosen 5.5 and organic modifier Acetonitrile.At pH 5.5 Ammonium Acetate suitable for mobile phase buffer preparation and for reducing peak tailing TEA was added and buffer organic(ACN) ratio 68:32v/v optimized for better separation at the flow rate 1.0 mL/min. UV detection at 238 nm and 271 nm for AML and VAL, respectively with injection volume of 20 µL and ambient temperature (30 °C) for the column were found to be best for analysis .
As AML was found to be very sensitive drug compare to VAL, when VAL shows countable degradation at the time AML was found to be degrade completely in most of stress conditions. To improve the accuracy of proposed method, degradation condition for both drugs were achieve separately combined dosage forms to get 10 to 30 % degradation.

Acidic conditions

For AML, standard solution was kept in 1M HCl for 1 h at room temperature, at 70 °C in water bath and at 100 °C in oven but no degradation was found. After number of trials

13 % degradation was achieved in the solution which was refluxed for 20 min in 1M HCl. The same condition was applied for sample in which 80 % degradation was found, so stress condition was reduced to 0.1M HCl and refluxed for 1 h in which 12% degradation was achieved.
For VAL, initially standard solution was kept in 0.1M HCl for 1 h at 70 °C on water bath, 30 min in reflux but no significant degradation was found. So, again standard solution was kept in 0.5M HCl and refluxed for 1 h in which only 5 % degradation was found. In next trial, sample was further refluxed for 6 h in which 10 % degradation was found. The same condition was applied to sample solution in that degradation was very high so time was reduced to 2 h in which 10 % degradation was achieved.

Alkali conditions
For AML, initially standard solution was kept in 0.1M NaOH for 1 h at room temperature, 70 °C in water bath and at 100°C in oven but no sufficient degradation was found. So again standard solution was refluxed in 0.1M NaOH for 45 min in which 16% degradation was achieved for standard solution. Then same condition was applied for sample solution but AML show remarka ble degradation so time was reduced to 20 min and 25% degradation was achieved.
For VAL, initially standard solution was kept in 0.5M NaOH for 1 h at 70 °C on water bath and under refluxed condition for 2 h but no degradation was found. So again solution was further refluxed for 6, 12, 24 h in which no degradation was found that indicate VAL was stable in alkali same is true for sample solution.

Oxidation conditions
For AML, initially standard solution was kept in 10 % H2O2 for 1 h at room temperature, 30 min in oven and on reflux for 30 min but no sufficient degradation was found. So again solution was refluxed in 30 % H2O2 for 20 min in which 28 % degradation was achieved. Then same condition was applied for sample solution but AML show remarkable degradation so, concentration of H2O2 was reduced to 10 in which 22 % degradation was found.
For VAL, initially sample solution was kept in 30 % H2O2 for 1 hr at 70 °C on water bath, 1 h at 100°C in oven and refluxed for 30 min but and no degradation was found so again solution was kept in 30 % H2O2 and refluxed for 2 h in which 11 % degradation was found.
The sample solution was also kept under same condition in that VAL shows high degradation so time was reduced to 45 min in which 13 % degradation was achieved.
Both thermal and photo degradation were performed and no degradation for both sample was found. The % degradation in stress condition for sample and standard with peak purity results were given in Table 1 & 1A.

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 peak area evaluated using six replicates of VAL (160 μg/mL) and AML (10 μg /mL).The intra- and inter-day variation for the determination of VAL and AML were evaluated and found coefficient of variation (CV) values of within-day and day-to-day variations for VAL and AML revealed that the proposed method is precise (Table 3). Accuracy of method was checked by a recovery study using the placebo addition method at 3 different concentration levels, i.e., a multilevel recovery study. The standards of for both drugs were spiked with 50, 100, and 150% with placebo, and the mixtures were analyzed by proposed method. Results of the recovery study are shown in Table 4 & 4A. The method was found to be robust even with small variations in flow rate (±0.2 mL/min), pH (±0.2 units) and concentration of acetonitrile (±10%) in the mobile phase as there was no significant difference in peak area and retention time.

Applicability of the Developed Method to Marketed Formulations
The assay results of VAL and AML in tablet dosage forms were comparable with the value claimed on the label. The obtained results, presented in Table 5, indicated the suitability of the method for routine analysis of VAL and AML from their combination drug products.

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 VAL and AML 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 VAL and AML in their combined dosage form.

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

Stress Condition	Drug Product		
	% Net degradation	purity Flag	Peak purity
Refluxed with 0.1M HCl solution for 1hrs	12.08	No	Pass
Refluxed with 0.1M NaOH solution for 20mins	24.98	No	Pass
Refluxed with 10% Hydrogen peroxide (H2O2) for 20 mins	21.87	No	Pass
Dry heated at 105° C for about 72 hrs	28.18	No	Pass
Exposed to Visible light for NLT 1.2 million lux hours	5	No	Pass

Table 1A: Results of forced degradation study using the proposed method. For Valsartan:

Stress Condition	Drug Product		
	% Net degradation	purity Flag	Peak purity
Refluxed with 0.5M HCl solution for 2hrs	10.23	No	Pass
Refluxed with 0.5M NaOH solution for 12hrs	0.12	No	Pass
Refluxed with 30% Hydrogen peroxide (H2O2) for 45 mins	10.87	No	Pass
Dry heated at 105° C for about 72 hrs	0.23	No	Pass
Exposed to Visible light for NLT 1.2 million lux hours	0.78	No	Pass

Table 2: Regression characteristics and system suitability parameter of proposed RP-HPLC method

Parameter	Val	Aml
Retention time (min)	1.98±0.18	4.03±0.13
Tailing factor	1.08±0.31	1.09±0.25
Asymmetry	1.06±0.71	1.11±0.65
Theoretical plates	53628±0.37	37493±0.34
Linearity range (µg/ml)	1.6-240	1-20
Regression equation (y*=a+bc)		
Slope (b)	15117±673.75	34684±528.64
Intercelpt (a)	28516±441.19	-2773.5±198.61
Correlation coefficient (r)	0.9998	0.9997

Table 3: Intra and Inter-day precision data of Proposed RP-HPLC method
Intra-day precision

S. No.	Amlodipine peak Area	Valsartan peak Area
1	429629	3006582
2	430381	3009518

3	431308	3013094
4	430768	3019260
5	431627	3022773
6	428967	3009867
Aveage	430447	3013516
%CV	0.23	0.21

Inter-day precision

S. No.	Amlodipine peak Area	Valsartan peak Area
1	427654	3012456
2	428987	3012045
3	429876	3017049
4	430897	3002345
5	429876	3021983
6	428765	3008967
Aveage	429943	3012474
%CV	0.23	0.21

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

Spike level	'µg/mL' added	'µg/mL' found (recovered)	Mean %Recovery
50%	79.74	80.45	100.89
100%	159.43	160.19	100.48
150%	239.45	239.87	100.18

n=Number of determinations

Table 4A: Recovery data for proposed RP-HPLC method (n=3)
For Amlodipine:

Spike level	'µg/mL' added	'µg/mL' found (recovered)	Mean %Recovery
50%	5.16	5.22	101.16
100%	10.54	10.45	99.15
150%	15.78	15.67	99.30

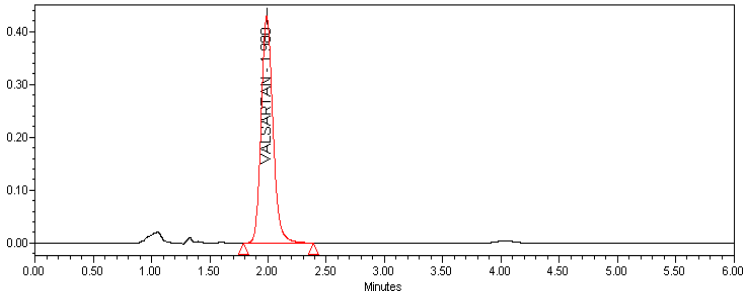
n=Number of determinations

Table 5: Applicability of the Developed Method to Marketed Formulations (n=5)

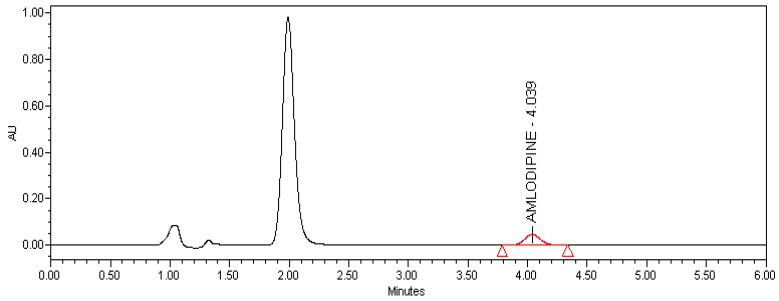
Fomulations	Labeled amount mg		Amount found (mg)		%Assay	
	AML	VAL	AML	VAL	AML	VAL
Set-1	10	160	9.96	159.67	99.6	99.79
Set-2	10	160	10.13	159.98	101.3	99.9

n=Number of determinations

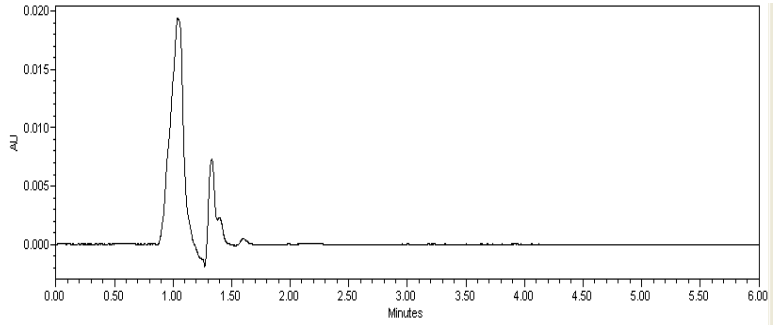
Typical chromatogram for valsartan at 271 nm



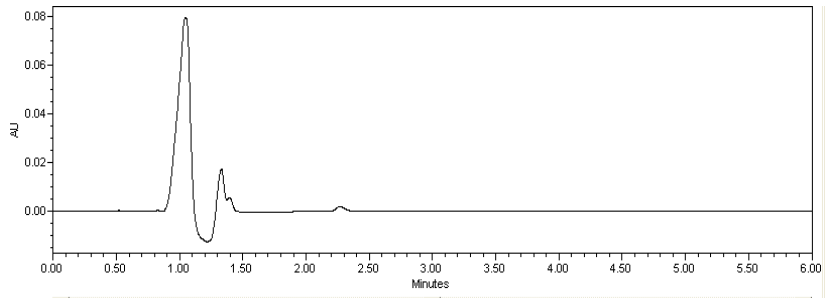
Typical chromatogram for Amlodipine at 238 nm



Typical chromatogram for Blank at 271 nm



Typical chromatogram for Blank at 238 nm



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