

Research Article

New Stability Indicating Method for Quantification of Impurities in Amlodipine and Valsartan Tablets by Validated HPLC

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A stability indicating LC method was developed for simultaneous determination of amlodipine and valsartan in pharmaceutical dosage form. Efficient chromatographic separation was achieved on C₈ stationary phase with simple combination of mobile phase-A (70 : 20 : 10 v/v/v of water : acetonitrile : methanol with 2 mL of Octylamine adjusted the pH to 2.50 + 0.05 with orthophosphoric acid) and mobile phase-B (Acetonitrile) delivered in gradient mode. Quantification was carried out using ultraviolet detection at 240 nm at flow rate of 1.0 mL/min with Injection Volume of 100 μL and ambient column temperature. This method was capable to detect both the drug components of Amlodipine and Valsartan in presence of their degradation products (Amlodipine Impurity-A and Valsartan Impurity-B) with the detection level of 0.05%. Amlodipine/Valsartan and their combination drug product were exposed to thermal studies, photolytic, hydrolytic and oxidative stress conditions, and samples analysed. Peak homogeneity data of Amlodipine and Valsartan is obtained using PDA detector, demonstrating the specificity. The method shows excellent linearity over range of 0.05–2.0% for Amlodipine; Amlodipine Impurity-A and 0.05–1.0% for Valsartan and Valsartan Impurity-B. The correlation coefficient for Amlodipine and Valsartan are 0.9999. The proposed method was found to be suitable and accurate for quantitative determination and the stability study of Amlodipine and Valsartan in pharmaceutical preparations.

1. Introduction

The amlodipine besylate component of amlodipine/valsartan tablets is chemically described as 3-ethyl-5-methyl (±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridinedicarboxylate, monobenzenesulphonate [1]. Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the movement of calcium ions into vascular smooth muscle cells and cardiac muscle cells.

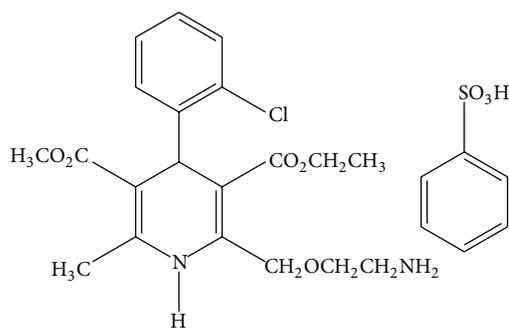
Amlodipine besylate has empirical formula of C₂₀H₂₅ClN₂O₅·C₆H₆O₃S, and its molecular weight is 567.1, and its structural formula is shown in Scheme 1.

Valsartan is a nonpeptide, orally active and specific angiotensin II antagonist acting on the AT₁ receptor subtype.

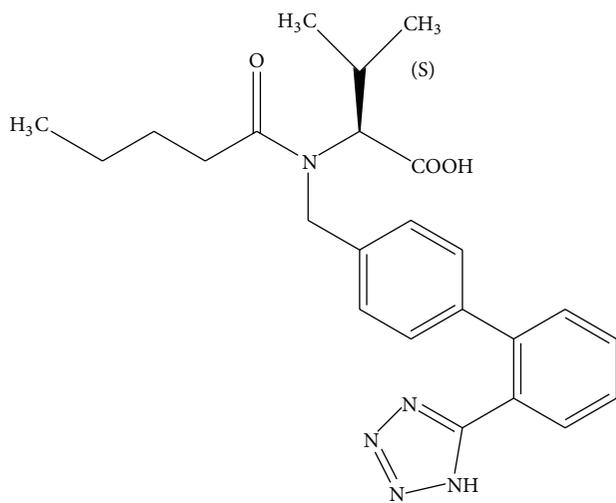
valsartan is a white to practically white fine powder, soluble in ethanol and methanol and slightly soluble in water. Valsartan's chemical name is N-(1-oxopentyl)-N-[[[2'-(1H-tetrazol-5-yl)[1, 1'-biphenyl]-4-yl] methyl]-L-valine [1].

Its empirical formula is C₂₄H₂₉N₅O₃, molecular weight is 435.5, and its structural formula is shown in Scheme 2.

Amlodipine/valsartan Tablets Brand name called as Exforge. Exforge contains 2 of the no. 1 prescribed high blood pressure medicines of their classes: amlodipine, a calcium channel blocker (CCB), and valsartan, an angiotensin receptor blocker (ARB). In clinical studies, EXFORGE was proven to be more effective in lowering high blood pressure than either of its components alone [2]. Amlodipine and valsartan are official in USP [3] but their combination drug product is not official in any pharmacopoeia. Exforge was proven to



SCHEME 1: Amlodipine chemical structure.



SCHEME 2: Valsartan chemical structure.

significantly lower high blood pressure in adults, regardless of age or gender. The blood pressure-lowering effects of Exforge have been studied in several clinical trials that included more than 5,000 patients with high blood pressure.

Exforge is available in a variety of dosage combinations that can be adjusted to best fit your needs. The dosage combinations are given as 5/160 mg, 5/320 mg, 10/160 mg, and 10/320 mg.

Amlodipine and valsartan tablets are developed in reference to (brand) Exforge tablets. Similar invagen has 4 strengths, namely, 5/160 mg, 10/160 mg; 5/320 mg, and 10/320 mg with the approximate tablet weights being 475 mg, 603 mg, 835 mg, and 950 mg, respectively.

The inactive ingredients and colorants for all strengths are standard excipients used in generic products.

Since this combination product has not been published in USP, a new method has been developed and validated as per ICH guideline and monitoring of these impurities with good separation of peaks and quantification of impurities in amlodipine/valsartan tablets as shown in Scheme 3.

Stability testing forming an important part of the process of drug product testing is to provide evidence on how 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 life to

TABLE 1: Elution: gradient.

Time (min)	Composition of mobile phase		Elution
	% A	% B	
0.01	100	0	Isocratic
20	100	0	Isocratic
60	40	60	Linear gradient
62	100	0	Reequilibration
75	100	0	Reequilibration

be established. The two main aspects of drug products that play an important role in shelf life determinations are assay of active drug and degradants generated during the stability study. Stability-indicating methods have been reported for assays of various drugs in drug products containing only one active drug substance. Only few stability-indicating methods are reported for the impurity assay of combining drug products containing two or more active drug substances. The objective of this work was to develop an analytical LC procedure, which would serve as stability-indicating impurity assay method for combining drug products of amlodipine and valsartan.

The literature survey reveals that several methods were reported for the individual estimation of amlodipine and valsartan. Various methods use HPLC [4–6], RP-HPLC [7, 8], HPTLC [9, 10], LC-MS [11], and LCMS/MS [12] and simultaneous UV spectrophotometric.

Methods [13, 14] are reported for the estimation of amlodipine alone or in combination with other antihypertensive agents. Methods such as HPLC [15–17], LC-MS [18–20], protein precipitation [21], capillary electrophoresis [22], and simultaneous UV spectrophotometer methods [23, 24] are reported for estimation of valsartan alone or in combination with other agents. None of the reported analytical procedures describe a stability indicating method for simultaneous determination of amlodipine and valsartan in combined pharmaceutical dosage form in the presence of their degradates.

If the reported individual methods are applied for the analysis of the tablets containing amlodipine and valsartan it would require UPLC to have shorter runtime and it is not possible for all firms to afford the same, and the method would not be rapid, less expensive, or economical, whereas the simultaneous determination of the ingredients of the tablets would be rapid, stability indicative, and also economical and can be afforded by all.

In the present study attempts were made to develop a rapid, economical, precise, and accurate method for the simultaneous estimation of the ingredients of this combination in the presence of their degradants.

2. Experimental

2.1. Chemicals and Reagents. Samples of amlodipine impurity-A and the valsartan impurity-B were synthesized and characterized at Hetero Drugs and Hetero Labs Limited, India. HPLC grade acetonitrile and methanol were procured

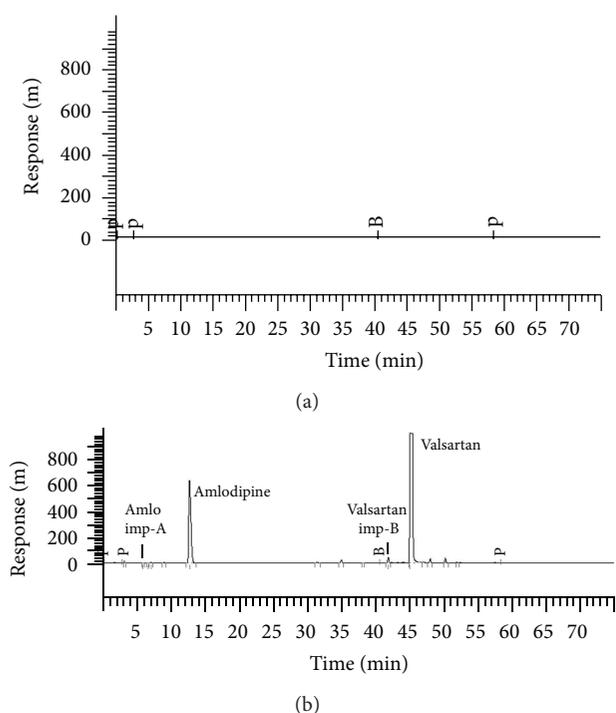
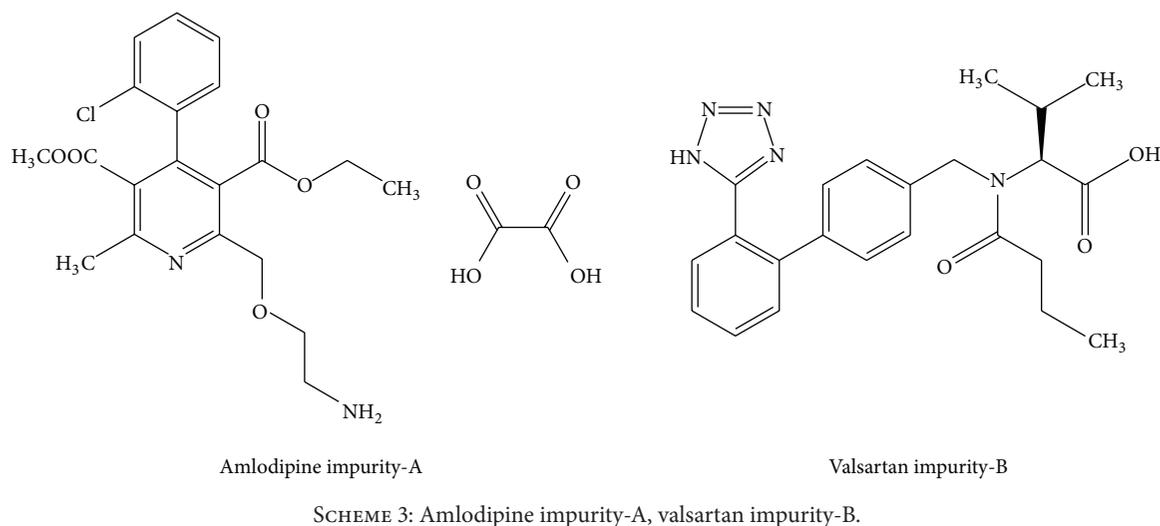


FIGURE 1: Chromatogram of Placebo and sample of 5/320 mg on acid degradation.

from Honeywell: Burdick & Jackson, muskegon, MI, and analytical grade octylamine was procured from Sigma Aldrich Company. High purity water was generated in-house from Siemens water purification system.

2.1.1. Chromatographic Conditions. The chromatographic system used was Shimadzu LC 2010 HPLC system comprised of degasser, quaternary pump, autoinjector, column compartment, and UV detector, and the system was controlled through EZ chrome software. Zorbax SB C8 column (i.d. 4.6 × 150 mm, 3.5 μm, Advance Chromatography, USA),

maintained at 25°C using a column oven, was eluted with mobile phase at the flow rate of 1.0 mL min⁻¹ with gradient program (see Table 1).

Mobile Phase A: Preparation involved transfer of 700 mL of DI Water, 200 mL of Acetonitrile and 100 mL of Methanol into suitable container, with addition of 2.0 mL of Octylamine and mixed well. Adjusted the pH to 2.50 ± 0.05 using Ortho Phosphoric acid. Filtered through 0.45 μm nylon membrane filter and degassed. **Mobile Phase B:** Acetonitrile.

Measurements were made with injection volume 100 μL and ultraviolet (UV) detection at 240 nm.

For standard and sample solution preparation were done using the diluents consisting of Methanol and DI water in the ratio of 50 : 50 v/v.

For analysis of forced degradation samples, the photodiode array detector (Model no. 2998) and Empower Software were used in scan mode with a scan range of 200–400 nm. The peak homogeneity was expressed in terms of peak purity and was obtained directly from the spectral analysis report using the previously-mentioned software.

2.1.2. Standard Stock Solutions. Standard solutions were prepared by dissolving the drugs in the diluent and diluting them to the desired concentration.

2.1.3. Amlodipine. 17.36 mg amlodipine standard (99.8%) was accurately weighed, transferred into a 250 mL volumetric flask, and dissolved with diluent.

2.1.4. Valsartan. 320.0 mg valsartan standard (99.3%) was accurately weighed, transferred into a 100 mL volumetric flask, and dissolved with diluent.

2.1.5. Low-Level Standard Preparation. The concentration of Low-level standard preparation contains 0.0003472 mg/mL of amlodipine besylate and 0.016 mg/mL of valsartan.

2.1.6. Detectability Level Standard Preparation. The concentration of detectability level standard preparation

TABLE 2: Results of analysis of forced degradation study samples using the proposed method, indicating percentage of degradation and peak purity of amlodipine and valsartan.

(a)

Degradation in	Degradation sample	Amlodipine imp-A (%)	Valsartan imp-B (%)	Maximum individual unknown impurity (%)	Total impurities (%)
As such sample	Placebo + amlodipine and valsartan	0.12	0.07	BRL	0.24
1N HCl (5 mL)	Placebo + amlodipine and valsartan	0.11	0.07	0.06	0.34
1N NaOH (5 mL)	Placebo + amlodipine and valsartan	0.11	0.07	0.06	0.34
30% H ₂ O ₂ (5 mL)	Placebo + amlodipine and valsartan	5.16	ND	0.31	6.35
UV light	Placebo + amlodipine and valsartan	0.10	0.07	0.06	0.28
Thermal condition at 80°C	Placebo + amlodipine and valsartan	0.11	0.07	0.06	0.34

ND: not detected; BRL: below reporting level (BRL = 0.05%).

(b)

Degradation samples	Peak area	Peak area	Retention time (min)	Purity angle	Purity threshold	Match angle	Match threshold
Placebo + drug degradation in 5 mL of 1N HCl	Amlodipine	2598903	12.30	0.236	0.432	0.138	1.159
	Valsartan	2943627	40.92	0.050	0.291	0.038	1.025
Placebo + drug degradation in 5 mL of 1N NaOH	Amlodipine	2599981	12.36	0.233	0.448	0.128	1.164
	Valsartan	2938404	40.91	0.047	0.287	0.042	1.025
Placebo + drug degradation in 5 mL of 30% H ₂ O ₂	Amlodipine	2483045	12.38	0.223	0.431	0.138	1.168
	Valsartan	2891144	40.92	0.063	0.319	0.055	1.038
Placebo + drug degradation in UV light	Amlodipine	2586948	12.05	0.234	0.438	0.220	1.162
	Valsartan	2933545	40.76	0.059	0.303	0.109	1.031
Placebo + drug degradation in thermal condition at 80°C	Amlodipine	2602008	12.46	0.247	0.448	0.193	1.161
	Valsartan	2924035	40.88	0.049	0.288	0.051	1.025

contains 0.00003472 mg/mL of amlodipine besylate and 0.0016 mg/mL of valsartan.

2.1.7. Impurity Stock Solution. Transfer accurately Weighed 1.80 mg of Amlodipine Impurity-A Standard in to 100 mL of volumetric flask, and dissolved with diluent.

2.1.8. Identification Solution. Transfer accurately Weighed 6.94 mg of Amlodipine Standard in to 100 mL of volumetric flask, and added 5.0 mL of the above impurity stock solution dissolved with diluent. The concentration of identification solution contains 0.0009 mg/mL of amlodipine besylate impurity-A and 0.0694 mg/mL of amlodipine besylate.

2.1.9. Preparation of Sample. Twenty tablets were weighed for average weight and finely powdered. A quantity of powder equivalent to 320.0 mg of valsartan was transferred into a 100 mL volumetric flask. To this flask, 75 mL of diluent was added, and the solution was sonicated for about 15 min with

intermittent shaking and with mechanical shaking for about 15 min. The solution was cooled to ambient temperature. Then the volume was made up with diluent and centrifuged at 10,000 rpm for about 15 min. Then the solution was used for injection.

2.1.10. Analytical Method Validation. The developed chromatographic method was validated for selectivity, linearity, range, precision, accuracy, sensitivity, robustness, and system suitability.

2.1.11. Selectivity/Specificity. Selectivity of the developed method was assessed by performing forced degradation studies. According to ICH stress testing of the drug substance can help the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedure used. Photo stability testing should be an integral part of stress testing. The standard conditions for photo stability testing are described in ICH Q1B. The specificity of the developed

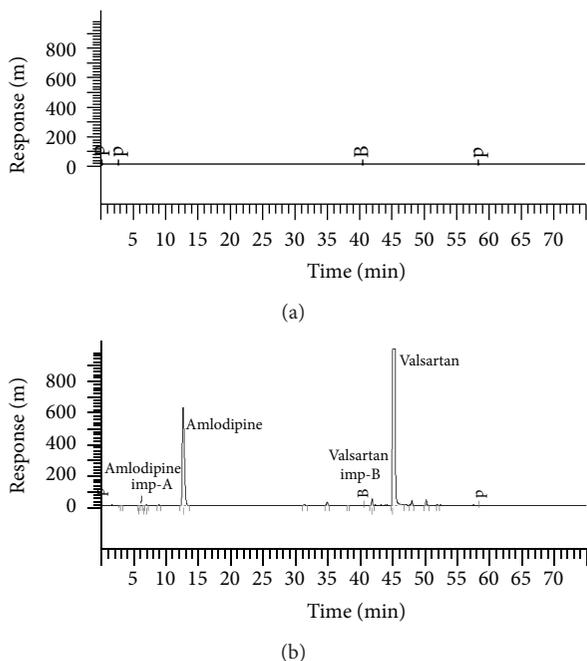


FIGURE 2: Chromatogram of Placebo and sample of 5/320 mg on base degradation.

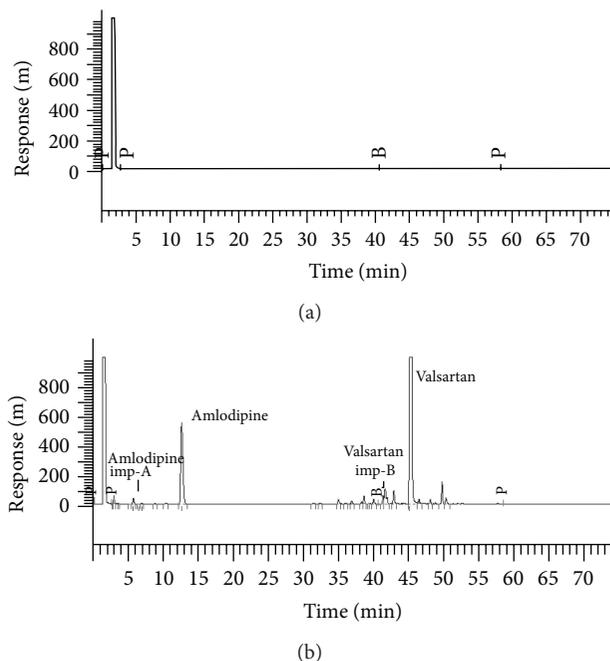


FIGURE 3: Chromatogram of placebo and sample of 5/320 mg on oxidation.

LC method for amlodipine and valsartan was determined in the presence of its related compounds amlodipine impurity-A, valsartan impurity-B. The stress conditions employed for degradation study include photolysis (ICH Q1B), thermal studies (80°C), acid hydrolysis (1N HCl), base hydrolysis (1N NaOH) and oxidation ($30\% \text{H}_2\text{O}_2$). For thermal study period was 7 days at 80°C , whereas for acid, base, and oxidation it was 2 hours. The results of data are presented in Tables 2(a) and 2(b) and related chromatograms and spectra are represented in Figures 1 through 10.

3. Results and Discussion

3.1. Optimization of the Chromatographic Conditions. To develop the stability-indicating method different stationary phases like C18, CN, different mobile phases containing buffers like phosphate, ammonium acetate, and trifluoroacetic acid with different pH (3–5) and organic modifier (acetonitrile) were used.

Our objective of the chromatographic method development was to achieve a peak tailing factor <2 , signal to noise ratio should be above 10, theoretical plates should be above 1000 for amlodipine and valsartan, % RSD for 5 consecutive injection should be less than NMT 10.0%, and very good separation between amlodipine impurity-A, valsartan impurity-B along with drug peak amlodipine and valsartan.

The chromatographic separation was achieved using a Zorbax SB C8 column (i.d. 4.6-150 mm, $3.5 \mu\text{m}$). Changing the composition of mobile phase optimized the chromatographic method. Segregation of all of the 2 peaks (amlodipine and valsartan) was observed on any C18 or CN column, but

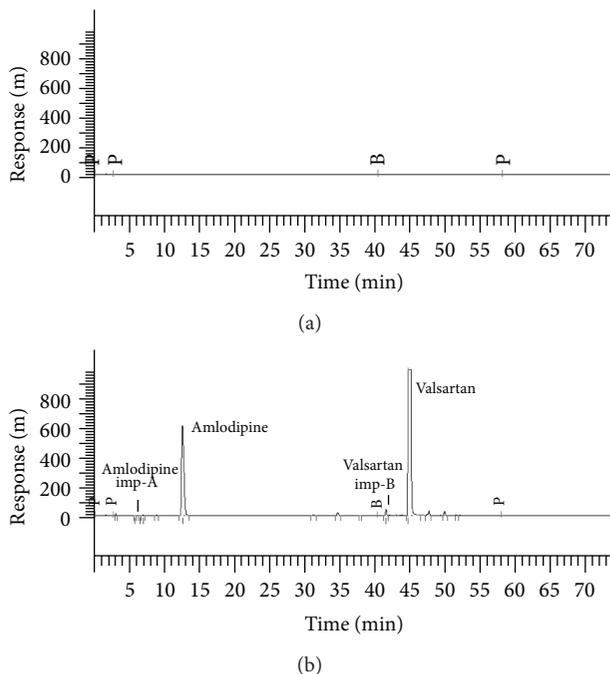


FIGURE 4: Chromatogram of placebo and sample of 5/320 mg on UV treatment.

it was difficult to separate both drug degradants on these columns (amlodipine impurity-A and valsartan impurity-B). The Zorbax SB C8 column shows better performance as compared to other columns.

From the development studies, it was determined that using mobile phase-A as water: acetonitrile: methanol in

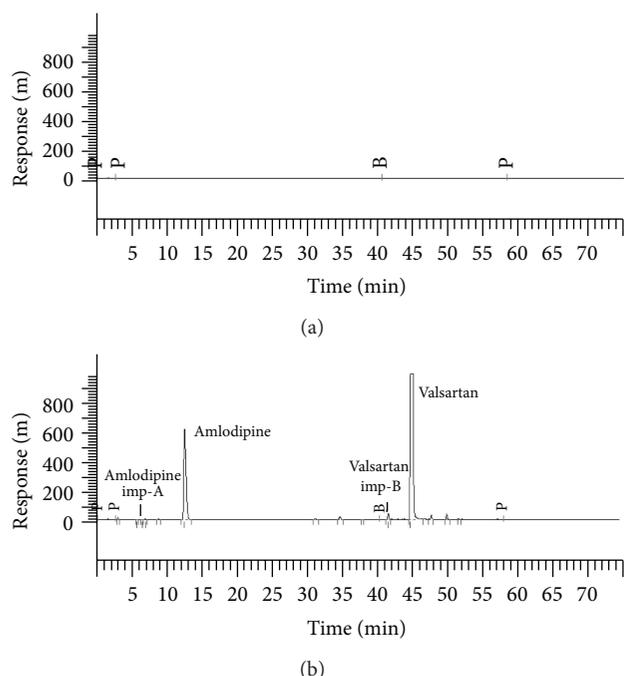


FIGURE 5: Chromatogram of placebo and sample of 5/320 mg on thermal treatment.

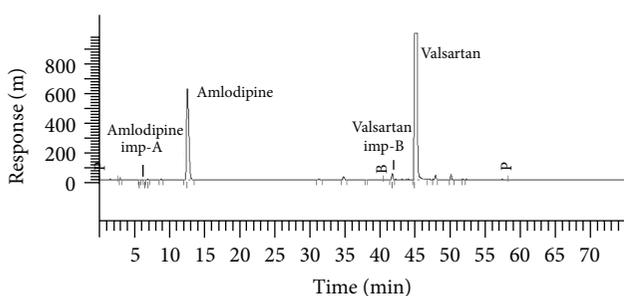


FIGURE 6

the ratio of 70:20:10 (v/v) with 2.0 mL of octylamine and adjusted the pH to 2.50 ± 0.05 using Ortho Phosphoric acid and Mobile phase-B as Acetonitrile with Gradient flow rate of 1.0 mL/min and ambient temperature, yielded good results. The analytes of this combination had adequate retentions, peak shape, less tailing, and more resolution between drug and its degradants, and the chromatographic analysis time was about 70 min. In optimized conditions amlodipine, valsartan, and their degradants were well separated. Typical retention times of amlodipine and valsartan were about 12.03 and 44.92 min.

Even the retention time looks little higher but the separations were achieved to greater extent and the methods prove stability indicating. Instead of regular HPLC if we use UFLC the retention time can be reduced to 20 minutes with the same suitability parameters, but when compared to cost efficiency all cannot afford UFLC.

During the initial forced degradation experiments, it was observed that oxidation was a fast reaction for amlodipine and valsartan tablets, and almost complete degradation

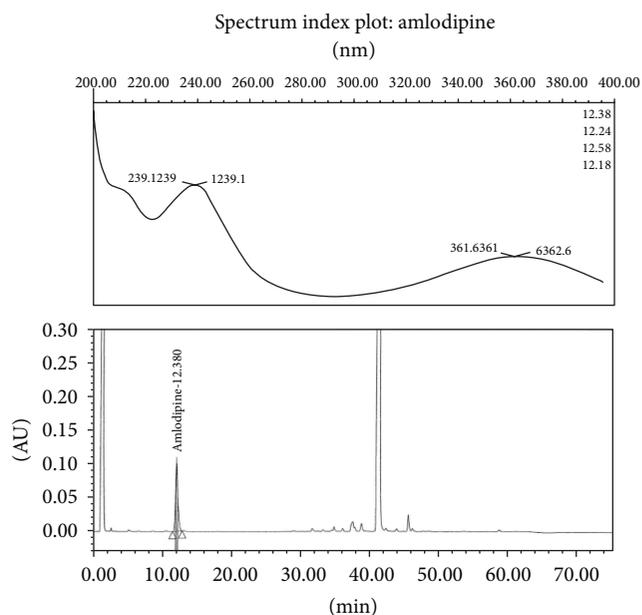


FIGURE 7

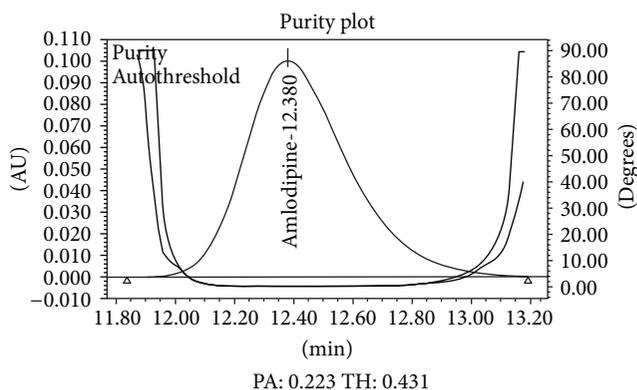


FIGURE 8

occurred when 5 mL of 30% H_2O_2 solution was used. Both drugs showed extensive degradation in alkali hydrolytic and oxidative condition and indicate homogeneous peaks and thus establishing the specificity of the impurity assay method.

3.2. Calibration and Linearity. Calibration curve obtained by the least square regression analysis between average peak area and concentration showed linear relationship with a regression coefficient of 0.999 over the calibration ranges tested.

The results of linearity and range obtained for the two potential impurities were tabulated. Linear calibration plot for this chromatographic method was obtained over the calibration ranges tested, that is, 0.05% to 1.0% for amlodipine impurity-A and 0.05% to 1.0% for valsartan impurity-B. The correlation coefficient obtained was greater than 0.999 for the two impurities and the major compounds amlodipine and valsartan (Figures 11 and 12). The method exhibited

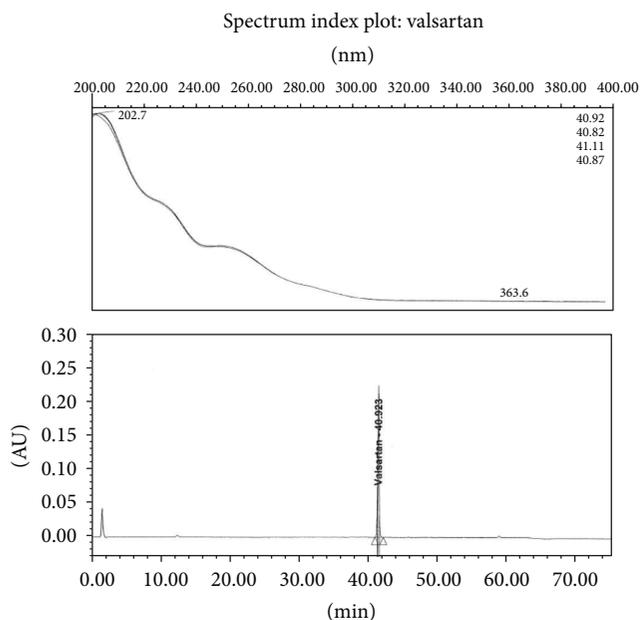


FIGURE 9

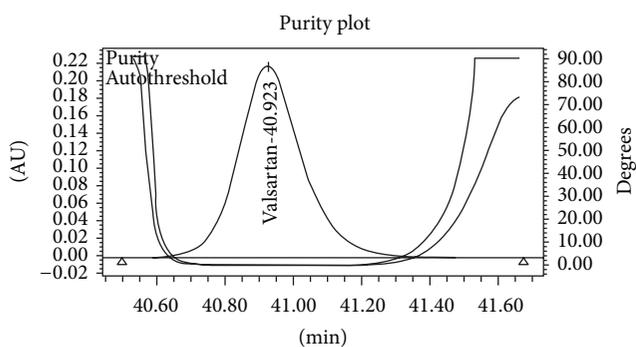


FIGURE 10

TABLE 3: (a) % RSD of six (6) replicate injections of each impurity should be less than 10.0, theoretical plates should be NLT 1000 and tailing factor should be NMT 2.0 for system precision. (b) % RSD of six (6) sample preparations for each impurity should be less than 10.0 for method precision.

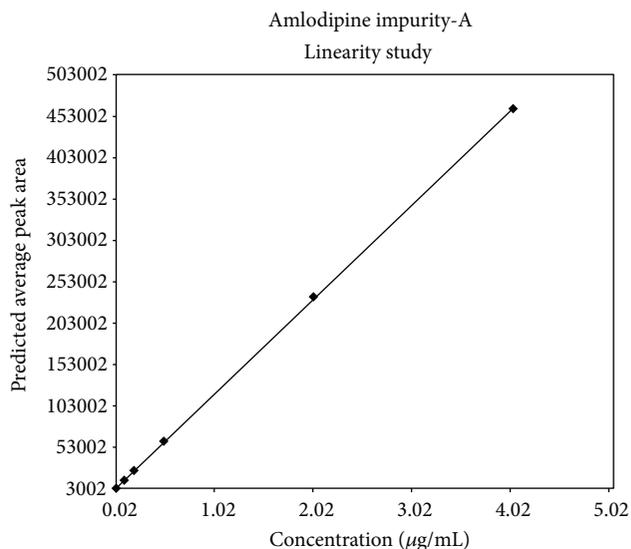
(a)

System precision			
Amlodipine		Valsartan	
% RSD	0.85	% RSD	0.89
Theoretical plates (N)	12460.23	Theoretical plates (N)	617503.71
Tailing factor (T)	1.034	Tailing factor (T)	1.045

(b)

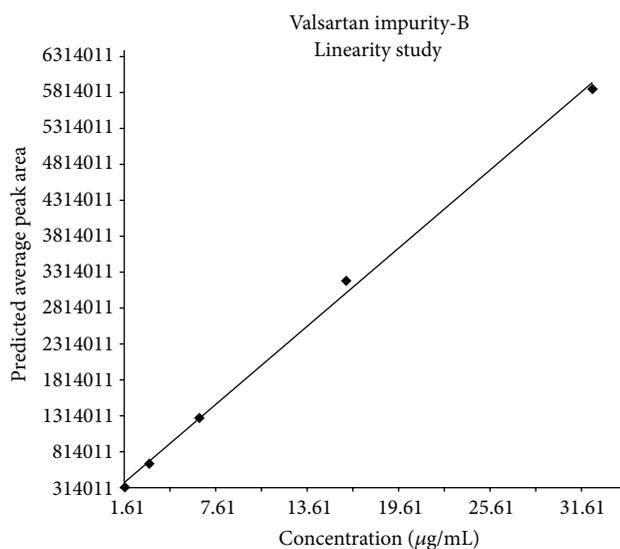
Method precision 5/320 mg			
Amlodipine impurity-A	1.06	Valsartan impurity-B	0.83
% RSD		% RSD	

good linearity with correlation coefficient values greater than 0.999.



◆ Observed average peak area
 — Predicted average peak area
 Slope (m) = 114059.040
 Intercept (c) = 1443.682
 Correlation coefficient (r) = 0.9999716

FIGURE 11

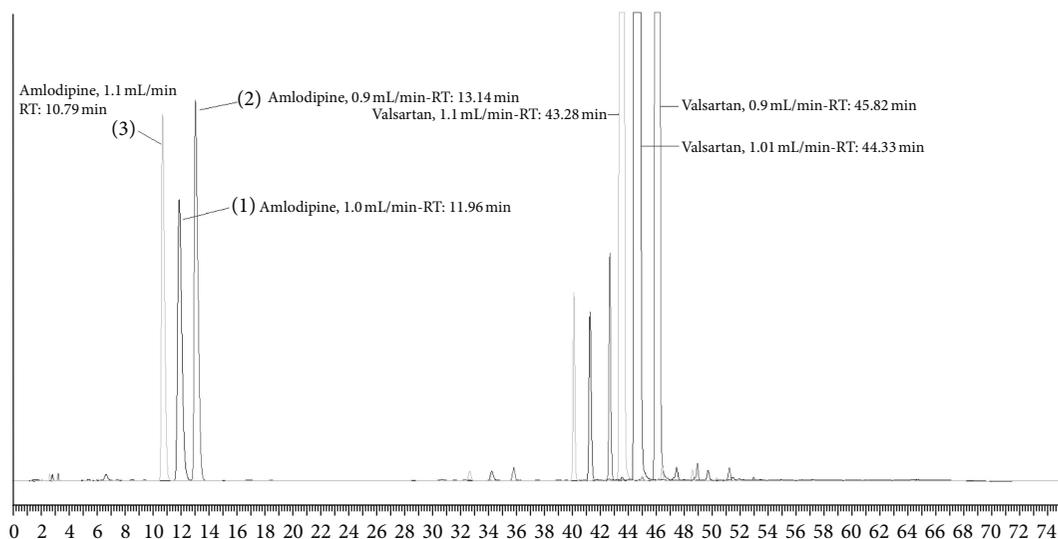


◆ Observed average peak area
 — Predicted average peak area
 Slope (m) = 181378.558
 Intercept (c) = 91687.060
 Correlation coefficient (r) = 0.9990036

FIGURE 12

3.3. Precision (Repeatability). The precision of the method was studied by determining the concentrations of each 0.85 and 0.89. The results of the precision study indicate that the method is reliable (RSD% < 10), in Tables 3(a) and 3(b).

3.4. Accuracy (Recovery Test). The percentage recovery was established for all the analytes throughout the range



- (1) Drug + 0.5% impurities, normal condition
 (2) Drug + 0.5% impurities, flow rate, 0.9 mL/min
 (3) Drug + 0.5% impurities, flow rate, 1.1 mL/min

FIGURE 13

TABLE 4

Concentration $\mu\text{g/mL}$	% Spiking level	Average peak area	% Recovery	Mean recovery %
Amlodipine imp-A				
0.0310	0.05	2806	94.0	101.5
0.3110	0.5	28745	96.3	
3.1100	2.0	121197	101.5	
Valsartan imp-B				
1.6475	0.05	339061	105.2	105.4
16.475	0.5	3398554	105.5	
164.75	1.0	6795594	105.4	

TABLE 5: S/N ratio of amlodipine, valsartan and composite impurities at LOQ (0.05%) level.

Component name	Concentration $\mu\text{g/mL}$	% RSD	Signal to noise ratio (S/N)
Amlodipine impurity-A	0.0253	5.43	75.672
Amlodipine	0.0346	0.70	100.156
Valsartan impurity-B	1.6145	0.71	9168.987
Valsartan	1.5900	0.66	9977.428

concentration as explained under linearity studies, and obtained results are tabulated later in Table 4.

4. Robustness

Robustness study was conducted by making small but deliberate changes in the optimized method parameters.

To determine robustness of the method, experimental conditions were purposely altered and chromatographic resolution between amlodipine and valsartan was evaluated.

The flow rate of the mobile phase was 1.0 mL min^{-1} . To study the effect of flow rate on the retention time of amlodipine and valsartan, it was changed to 0.9 mL/min and 1.1 mL/min . The effects of pH variation were studied at pH 2.40 and pH 2.60 instead of pH 2.50, while other mobile phase components were held constant.

At all conditions the relative retention time found for amlodipine impurity-A and valsartan impurity-B was found 0.46 and 0.93, respectively (see Figure 13).

4.1. Determination of Limit of Quantification. Prepared Amlodipine and Valsartan LOQ solution as per the method containing the concentration of about $0.0346 \mu\text{g/mL}$ of Amlodipine and $1.5900 \mu\text{g/mL}$ of Valsartan. Made five (5) replicate injections and calculated S/N ratio of 0.05% to establish LOQ (see Table 5).

5. Conclusion

The gradient RP-LC method developed for the analysis of binary mixtures of amlodipine and valsartan in their pharmaceutical preparations is precise, accurate, and with a reasonable run time. The method was fully validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating, separates degradants, and can be conveniently used by the quality control department to determine impurity assay of pharmaceutical preparations and also stability sample analysis.

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