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DEVELOPMENT OF VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND CANDESARTAN CILEXETIL FROM TABLET

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ABSTRACT

A simple, isocratic, rapid and accurate reversed phase high performance liquid chromatography method was developed for the quantitative determination of Candesartan Cilexetil and Amlodipine Besylate tablets. The chromatographic separation was achieved on Water Xterra R18, 150×4.6 mm, 3.5u (C18) using Mobile Phase A : ACN: Water: OPA (950:50:01) and Mobile Phase B: ACN: Water: OPA (50:950:01), and the λmax of Amlodipine Besylate was detected at 237nm, where Candesartan Cilexetil exhibits sufficient absorbance at 254 nm. The linear range for Candesartan Cilexetil and Amlodipine Besylate were (2.8-42ppm) and (6.4-96) was obtained with correlation coefficients ≥0.999 for each analyte. The retention time were found to be 4.2 and 8.5 min Candesartan Cilexetil and Amlodipine Besylate respectively. Candesartan Cilexetil and Amlodipine Besylate was subjected to stress conditions (hydrolysis (acid, base) oxidation, photolysis, thermal degradation and humidity degradation) and the stressed samples were analyzed by use of the method. The major degradation was observed in base and minor in acid, thermal, oxidation, humidity and photolysis. The forced degradation studies prove the stability indicating power of the method.

KEYWORDS: Amlodipine Besylate (AMLO) and Candesartan Cilexetil (CANDE), Method validation, RP- HPLC, C18, Degradation Studies.

INTRODUCTION

Candesartan is a angiotensin (II) receptor blocker is alone or with other anti-hypertensive agent to treat hypertension. 2-ethoxy-1-({4-[2-(2H-1,2,3,4-tetrazol-yl)phenyl] phenyl} methyl)-1H-1,3-benzodiazole-7-carboxylic acid. The chemical structure of Candesartan Cilexetil shown in Fig.1.Amlodipine is a calcium channek blocker, it acts as anti-angina agent. Chemically is 3-ethyl 5-methyl (4RS)-2- [(2-aminoethoxy) methyl]-4-(2chlorophenyl)- 6-methy l-1, 4dihydropyridine-3, 5-dicarboxylate benzene sulphonate, the chemical structure of Amlodipine Besylate shown in Fig.2.[1] As per literature review, several methods were developed in individual /plain drug but this combination one or two method was developed. Candesartan Cilexetil and Amlodipine Besylate was estimated by only few method UV spectroscopy,[2,3,5] HPLC.[4] Only one stability indicating RP-HPLC method. The aim of present work was to develop and validate a accurate, cost effective and precise UV spectroscopy by simultanious equation method, Q-absorbance ratio method, RP-HPLC method for determination Candesartan Cilexetil and Amlodipine Besylate.

MATERIALS AND METHODS

INSTRUMENTATION

Agilent HPLC 1200 series chromatography equipped with quaternary gradient pump reciprocating water-510, multiwavelength PDA detector water-2469 with automated injector and equipped with software empower pro, reserved phase column such as water xterra RP18, 150×4.6mm, 3.5µ particle size and Kromasil C18, 100×4.6mm, 5µ particle size used for chromatographic studies, THERMOLAB stability chamber (Model No. TS 00002008).

pH meter: Digital pH meter, Make Thermo 5.1.4

Stability Chamber: THERMOLAB, Model No. TS 00002008

Mobile phase: Mobile Phase A: ACN:Water:OPA(950:50:01)

Mobile Phase B: ACN:Water:OPA(50:950:01)

MATERIAL

Amlodipine Besylate and Candesartan Cilexetil bulk powder were gifted In- house Getz Pharm Research, Mumbai.

Acetonitrile (HPLC grade).

Methanol (HPLC grade).

Select of wavelength

Amlodipine besylate standard solution

An accurately weighed quantity of Amlodipine Besylate 13.90mg (equivalent to 10mg Amlodipine) transferred in 100 mL volumetric flask, dissolved in sufficient quantity of methanol, sonicated for 5minutes and volume was made up to the mark with methanol. A 1 mL of the above solution was further diluted to 10 ml with methanol. [Concentration 10ug/mL]

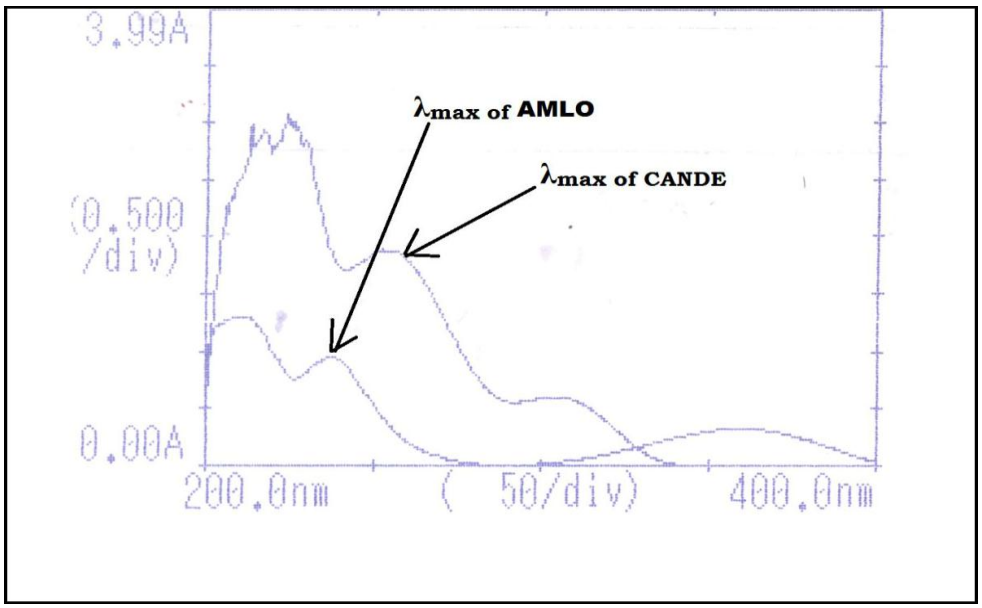
Candesartan cilexetil standard solution

An accurately weighed quantity of Candesartan cilexetil 10.00mg transferred in 100mL volumetric flask, dissolved in sufficient quantity of methanol, sonicated for 5minutes and volume was made up to the mark with methanol. A 1mL of the above solution was further diluted to 10 mL with methanol. [Concentration 10ug/mL].

Selection of wavelength

The standard solution AMLO and CANDE were scanned in the range from 200-400 nm with UV spectrophotometer using methanol as blank.

The overlain UV spectrum of Amlodipine Besylate and Candesartan cilexetil is as follow.



The study of Figure 1 shows that the λmax of Amlodipine Besylate was found to be 237 nm, where Candesartan Cilexetil exhibits sufficient absorbance at 254 nm and hence selected as detection wavelength for further experimentation.

Selection of mobile Phase and Chromatographic condition

Preparation of standard solution

Amlodipine besylate (AMLO) stock solution

Accurately weighed and transfer about 28mg of Amlodipine Besylate (equivalent to 20mg Amlodipine), in to 100mL volumetric flask. Add about 60 mL diluent, sonicate to dissolve. Make volume up to the mark with diluent and mix. (280µg/mL)

Candesartan cilexetil (CANDE) stock solution

Accurately weigh and transfer about 64 mg of Candesartan Cilexetil, in to 100mL volumetric flask. Add about 60 mL diluent, sonicate to dissolve. Make volume up to the mark with diluent and mix. (640µg/mL).

Mixed standard solution of amlodipine Besylate and Candesartan cilexetil

Separately pipette out 10 mL of Candesartan Cilexetil standard stock solution, 10 mL from Amlodipine Besylate standard stock solution into 100 mL volumetric flask, Make volume up to the mark with diluent.

(AMLO 28µg/mL, CANDE 64 µg/mL)

Optimization of chromatographic condition

Proper selection of method depends upon the nature of the sample (ionizable/ neutral molecule, its molecular weight and solubility). The drug wasselected in its present study is polar in nature therefore; reverse phase or ion exchange or ion pair chromatography method can also be used. Here, the RP-HPLC method was selected for the initial separation owing to its simplicity, suitability, ruggedness and its wider usage. In order to achieve the optimized chromatographic conditions to separate elute and quantify Candesartan cilexetil and Amlodipine besylate, one or two parameters were modified at each trial and chromatograms were recorded with all specified chromatographic conditions. Standard solutions were prepared in different diluent and various trials were taken for the selection of mobile phase, column, flow rate, wavelength, temperature, and pH are as follows.

Method development

A variety of mobile phases were investigated in the development of a RP-HPLC method for the analysis of Amlodipine Besylate and Candesartan Cilexetil. A mixture of ACN: Water: OPA (950:50:01) and ACN: Water: OPA (50:950:01) was found to be the most suitable mobile phase for ideal separation of Amlodipine Besylate and Candesartan Cilexetil. The

solvent mixture was filtered through a 0.22 µ PVDF filter and sonicated before use. It was pumped through the column at a flow rate of 1.5 mL/min. The detection of the drug was monitored 237 nm Amlodipine Besylate and 254 nm Candesartan Cilexetil. The run time was set at 20 min. Under the optimized chromatographic condition the retention time obtained for the drug was 4.236 min Amlodipine Besylate and 8.571 min Candesartan Cilexetil. A typical chromatogram showing the separation of the drug is as shown in Figure 2.

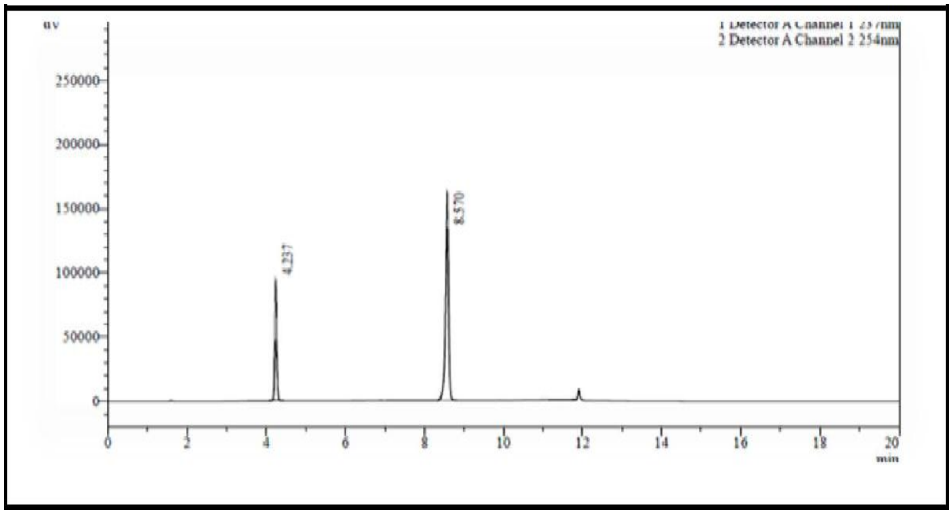


Fig. no. 2: A typical chromatogram showing the separation of the drug.

Final optimized chromatographic condition

Amlodipine besylate (AMLO) stock solution

Accurately weighed and transfer about 28 mg of Amlodipine Besylate (equivalent to 20 mg Amlodipine), into 100mL volumetric flask. Add about 60 mL diluent, sonicate to dissolve. Make volume up to the mark with diluent-I and mix. (280µg/mL).

Candesartan cilexetil (CANDE) stock solution

Accurately weighed and transfer about 64 mg of Candesartan Cilexetil, into 100mL volumetric flask. Add about 60 mL diluent, sonicate to dissolve. Make volume up to the mark with diluent-I and mix. (640µg/mL).

Mixed standard SOLUTION of AMLO and CANDE

10 mL of Candesartan Cilexetil standard stock solution was pipette and 10 mL of solution from Amlodipine Besylate standard stock solution was pipette into 100 mL volumetric flask, Make volume up to the mark with diluent-II.

Table no. 1: Optimized chromatographic conditions.

Parameter	Condition
Column	Water Xterra R18, 150×4.6 mm, 3.5u (C18)
Flow Rate	1.5 mL/min
Injection Volume	20 µl
Wavelength	237 nm Amlodipine Besylate 254 nm Candesartan Cilexetil
Column Temp.	40° C
Pump Flow	Gradient
Run Time	20 minutes
Mobile Phase	Mobile Phase A : ACN:Water:OPA(950:50:01) Mobile Phase B : ACN:Water:OPA(50:950:01)
Diluent	Diluent I - ACN:Water (30:70) Diluent II- Buffer pH 3: ACN (50:50)

System suitability study

The System suitability of the HPLC method was determined by making six replicate injections from freshly prepared standard solutions and analyzing each solute for their retention time, theoretical plates number (N) and tailing factors (T).

Procedure

The chromatographic conditions were set as per the optimized parameter and mobile phase was allowed to equilibrate with the stationary phase to get steady base line. After equilibration of column with mobile phase, five replicate injection of 20µL mixed solution where injected. The chromatogram recorded and the peak response i.e. peck area were measured. The result deplicated in Table 2.

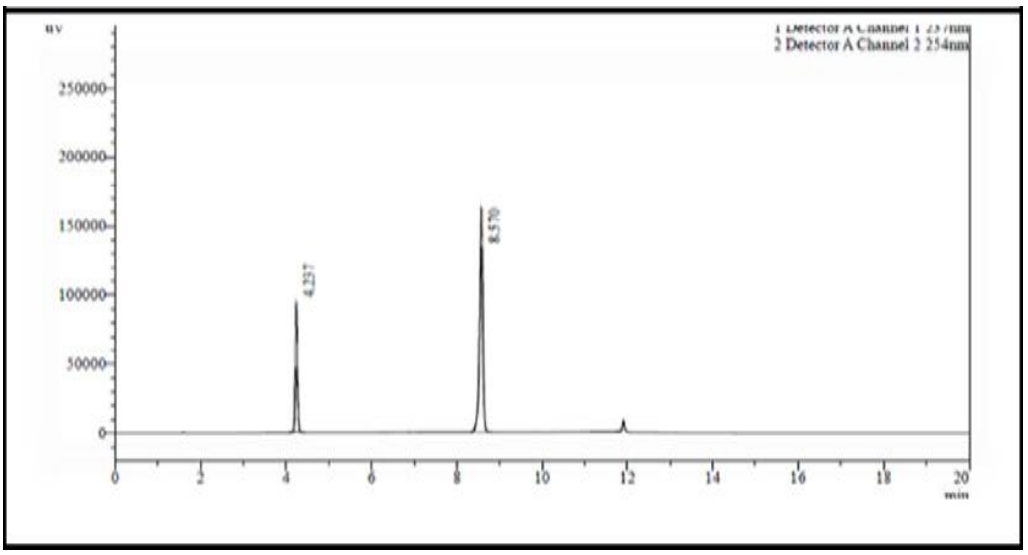


Fig. no. 3: Chromatogram for standard solution.

Table no. 2: Observation and result of system suitability parameters.

Sr. No.	Standard weight taken (mg)		AUC of AMLO (uV)	AUC of CANDE (uV)
	AMLO	CANDE		
1	28.32 mg	64.05 mg	373269	715717
2			373475	716569
3			373418	716420
4			373310	716295
5			373603	717050
Mean			373421.3333	716656.5
%RSD			0.03	0.10
Theoretical plate			20109	53113
Retention time (min)			4.236	8.571
Tailing factor			1.124	0.844

Study of Beer-Lambert's Law

Procedure accurately weighed and transfer about 28 mg of Amlodipine Besylate (≈ 20 mg Amlodipine) and 64 mg Candesartan cilexetil standard in 200mL volumetric flask. Add about 160mL of diluent, sonicate to dissolve. Make volume up to the mark with Diluent and mix. [Stock solution].

From the above stock solution 2.0mL 5.0mL, 8.0mL, 10.0mL, 12.0mL, and 15.0mL were transferred to the 100mL and 50mL of flask respectively.

The system was allowed to equilibrate for 30 minutes by passing the mobile phase. After equilibration, the injection of all test solution of amlodipine besylate and candesartan cilexetil in ascending order for different concentration levels were injected and chromatographed. The graphs of concentration of drug vs. area under curve were plotted for both the drug. The observation and result of drugs are summarized in the Table No. 3.

Table no. 3: Observation and Result of Linearity and Range.

% Level	Concentration(ppm)		Auc of amlo (Uv)	Auc of Cande (uV)
	Amlo	Cande		
	2.8	6.4	39305	72630
50	14	16	189678	364440
80	22.2	51.2	302630	581708
100	28	64	378477	727721
120	33.6	76.8	455502	876318
150	42	96	570498	1095225
% Y-intercept			1	0.9999
Intercept			824.15	4825
Slope			13542	11337
Regression			1.000	0.999

Recovery studies

It was carried out by standard addition method Preparation of sample Accurately weighed quantity of standard drug powder equivalent to 160 mg of CANDE and 70mg of AMLO was transferred to 100 mL volumetric flask and to it Placebo were added equivalent to 2199.2 mg, 60 mL of diluent-I was added and sonicate for 30 min, volume was made up to the mark with diluent-I and filtered through 0.45u PDFE filter. A 4.0mL portion of filtrate was further diluted to 200 mL with diluent –II to become 50%. Likewise 320 mg of CANDE and 140 mg of AMLO for 100% and 480 mg of AMLO and 210 mg of CANDE for 150% and placebo were added about 2199.2 mg for both 100% and 150% and dilution were carried out as such for 50%.

Table no. 4a: Observation and Result of Recovery for Amlodipine Besylate.

Level	Placebo taken in mg	mg of API added	Peck Area (vU)	Conc. Recovered	% Recovered	Mean
50%	2164.87	70.8	190100	10.323	101.8	102.2
			192637	10.461	103.2	
			189694	10.301	101.6	
100%	2198.74	140.8	377050	20.476	100.2	100.7
			370187	20.103	99.7	
			370185	20.141	99.2	
150%	2196.3	210.43	562117	30.526	101.7	101.3
			561003	30.465	101.1	
			567021	30.792	101.2	
Overall mean						101.16
Overall SD						1.1060
Overall % RSD						1.09%

Table no. 4b: Observation and Result of recovery for the candesartan cilexetil.

Level	Placebo taken in mg	mg of API added	Peck Area (vU)	Conc. Recovered	% Recovered	Mean
50%	2164.87	160.4	366223	31.969	100.8	100.8
			365444	31.901	100.4	
			370477	32.340	101.7	
100%	2198.74	320.48	736660	64.305	101.0	100.9
			732888	63.976	100.5	
			734965	64.157	100.8	
150%	2196.30	480.48	1097869	95.836	100.4	101.1
			1107213	96.652	101.3	
			1109697	96.869	101.5	
Overall mean						100.93
Overall SD						0.15275
Overall % RSD						0.15%

Stress degradation study by HPLC

In stress testing a drug substance or the drug product is exposed to an environment vigorous than normal sometime called as accelerated stability condition like high temperature, high humidity over the period of time, hydrolysis of drug with base or acid, and photo stability. Forced degradation studies are performed to get an idea of how drug substance or product degrades, degenerates and behave under changing condition, which helps in developing the stability indicating method of analysis (SIAM). The ICH guideline Q1A suggest the following condition to be employed.

A. Solution state analysis

a. Alkaline hydrolysis

b. Acidic hydrolysis

c. Oxidation hydrolysis

d. Neutral hydrolysis

B. Solid state analysis

a. Thermal studies (80°C)

b. Photochemical study

c. Humidity study (45°C±2°C and 75% RH)

Solution state stability

Acid hydrolysis

It was performed using 1N HCL. Accurately weigh and transfer 4 tablet in to a 100 mL of volumetric flask, add about 60 mL of diluent-I and sonicate for 30 minute with intermittent shaking. And add about 5mL of1NHCL and kept on bench top at room temperature or 12hr. neutralized this solution before dilution with1N NaOH. Allow to cool at room temperature. Make up the mark with diluent-I and mix. Filter through 0.45 u PTFE + prefilter discarding first few ml of filtrate. Further dilute 5 mL of this solution to 100 mL with diluent –II and mix. The treated sample was analyzed as per the proposed method. The sample were chromatographed using 20ul volume.

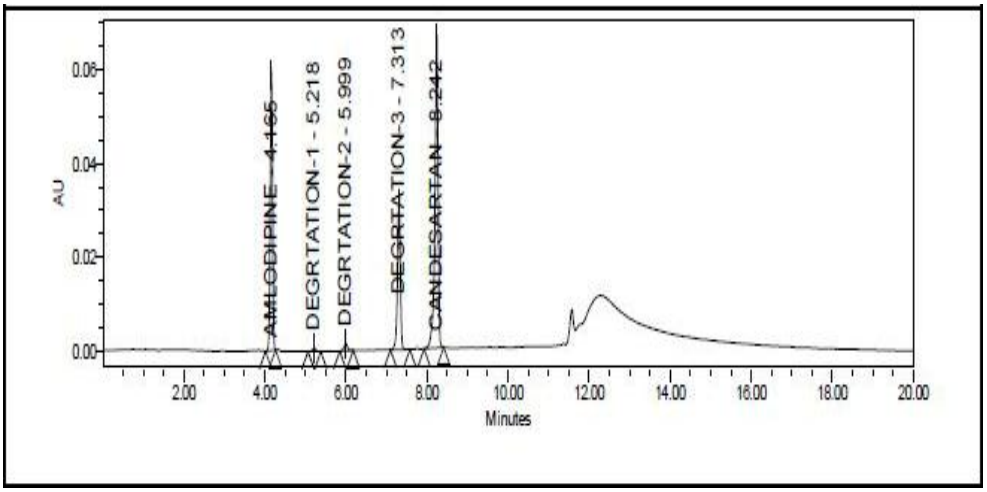


Fig. no.4: Chromatogram of acid treated sample (1N HCL).

Table no. 5: Result and observation for the acid degradation.

Sample	Peak area (Uv)	% assay	% degradation	Purity angle	Purity threshold	flag
AMLO	327382	91.3	7.96	0.708	1.252	NO
CANDE	605434	86.9	11.41	0.649	1.125	NO

Alkaline hydrolysis

It was performed using 1N NaOH

Accurately weigh and transfer 4 tablet in to a 100 mL of volumetric flask, add about 60 mL of diluent-I and sonicate for 30 minute with intermittent shaking. And add 5mLof1NNaOH and kept on bench top at room temperature for 1hr. neutralized this solution before dilution with1N HCL. Allow to cool at room temperature. Make up the volume with diluent-I and mix. Filter through 0.45 u PTFE + prefilter discarding first few ml of filtrate. Further dilute 5 mL of this solution to 100 mL with diluent –II and mix. The treated sample was analyzed as per the proposed method. The sample were chromatographed using 20ul volume.

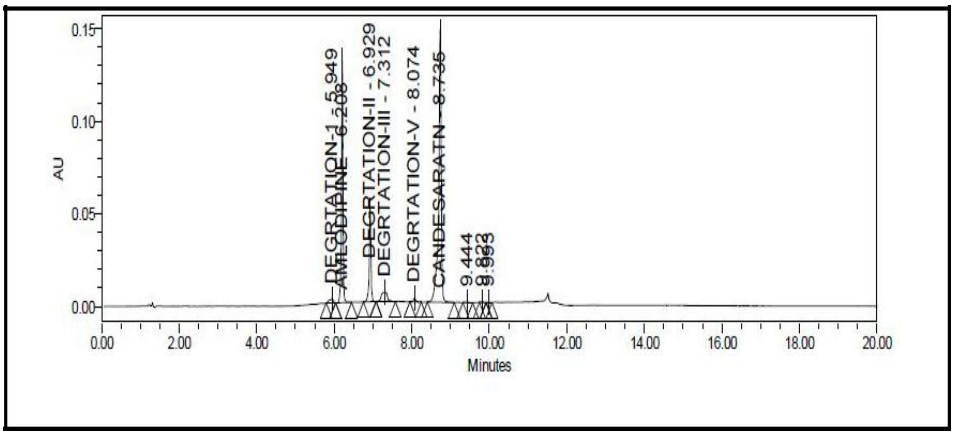


Fig. no. 5: Chromatogram of base treated sample (1N NaOH).

Table no. 6: Result and observation for the acid degradation.

Sample	Peak area (Uv)	% assay	% degradation	Purity angle	Purity threshold	flag
AMLO	327309	89.2	10.08	5.412	15.995	NO
CANDE	551776	77.9	20.36	0.879	1.371	NO

Oxidation studies

It was perforemed by using 30 % H2O2,

Accurately weigh and transfer 4 tablet in to a 100 mL of volumetric flask, add about 60 mL of diluent-I and sonicate for 30 minute with intermittent shaking. And add 5mL of 30% H2O2 and kept on bench top at room temperature for 6 hr. Make up the mark with diluent-I and mix. Filter through 0.45 u PTFE + prefilter discarding first few mLof filtrate. Further dilute 5 mL of this solution to 100 mL with diluent–II and mix. The treated sample was analyzed as per the proposed method. The sample were chromatographed using 20ul injection volume.

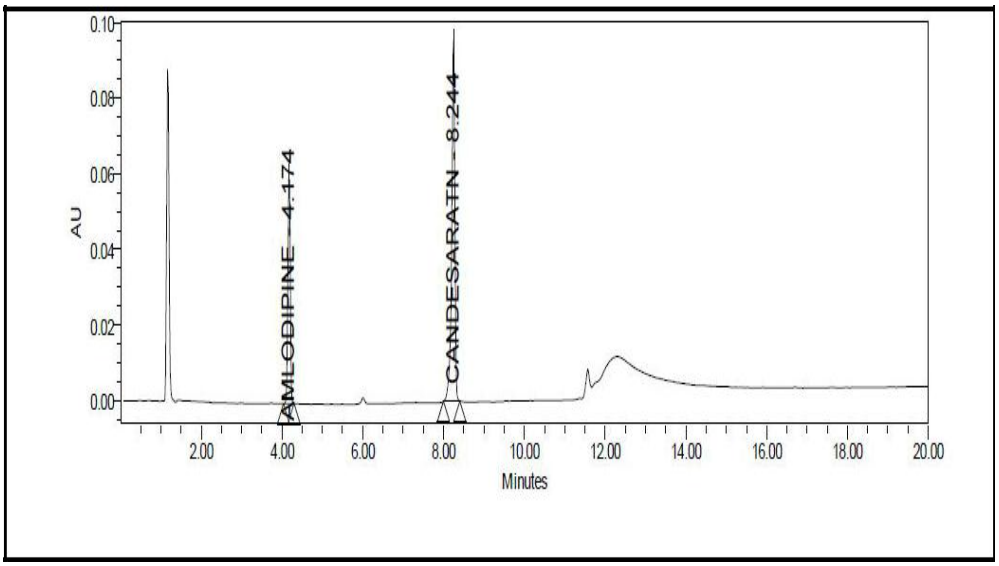


Fig. no. 6: Chromatogram of hydrogen peroxide treated.

Table no. 7: Result and observation for the Hydrogen peroxide.

Sample	Peak area (Uv)	% assay	% degradation	Purity angle	Purity threshold	flag
AMLO	329825	91.4	7.8	1.405	1.150	NO
CANDE	515305	74.9	23.2	0.668	1.091	NO

Solid state analysis

Thermal study

It was performed by heating on water bath at 70°

Accurately weigh and transfer 4 tablet in to a 100 mL of volumetric flask, add about 60 mL of diluent-I and sonicate for 30 minute with intermittent shaking. And place in water bath having temperature of about 70°C for about 12hr. After12 hr. make up the volume with diluent-I and mix. Filter through 0.45 u PTFE + prefilter discarding first few ml of filtrate. Further dilute 5 mL of this solution to 100 mL with diluent–II and mix. The treated sample was analyzed as per the proposed method. The sample were chromatographed using 20ul injection volume.

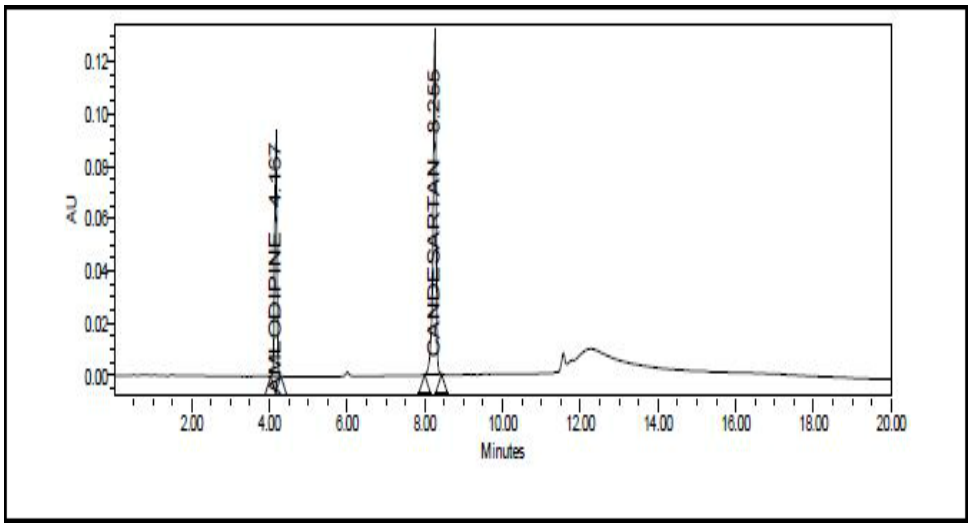


Fig. no. 7: Chromatogram of sample under thermal degradation.

Table No. 8: Result and observation for the thermal degradation.

Sample	Peak area (Uv)	% assay	% degradation	Purity angle	Purity threshold	flag
AMLO	328808	91.12	8.86	1.405	1.150	NO
CANDE	685599	99.66	-	0.668	1.091	NO

Humidity degradation

It was performed on 45°C/75% RH

Accurately weight and transfer about 4 Tablet in Petri dishes and kept in humidity chamber at 450C/75% RH for 12 days. On the day of analysis the exposed solid samples were withdrawn. The sample solution were prepared by following the general procedure as described in procedure for formulation. A 20µL volume of solution was injected.

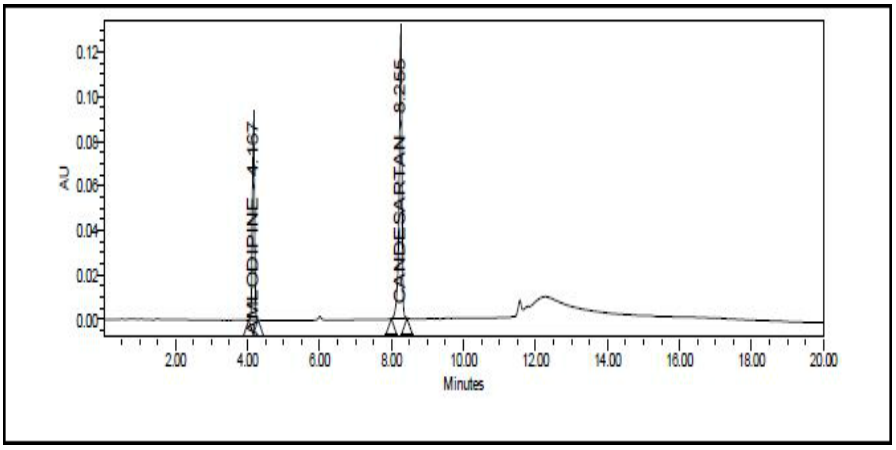


Fig. no. 8: Chromatogram of sample under humidity degradation.

Table No.9: Result and observation for the Humidity degradation.

Sample	Peak area (Uv)	% assay	% degradation	Purity angle	Purity threshold	flag
AMLO	320674	88.90	10.38	0.595	1.137	NO
CANDE	718854	104.49	-	0.649	1.125	NO

Photolytic degradation

It was performed in photolytic chamber

The tablet were placed in two petri plate one closed and one is open and exposed to the light providing an overall illumination of not less 1.2 million lux hour and an integrated near ultraviolet energy not less than 200 watt hours/ square meter for 12 days. Accurately weigh and transfer 4 tablet in to a 100 mL of volumetric flask, add about 60 mL of diluent-I and sonicate for 30 minute with intermittent shaking. Make up the mark with diluent-I and mix. Filter through 0.45 u PTFE + prefilter discarding first few ml of filtrate. Further dilute 5 mL of this solution to 100 mL with diluent –II and mix. The treated sample was analyzed as per the proposed method. The sample were chromatographed using 20ul volume. T.

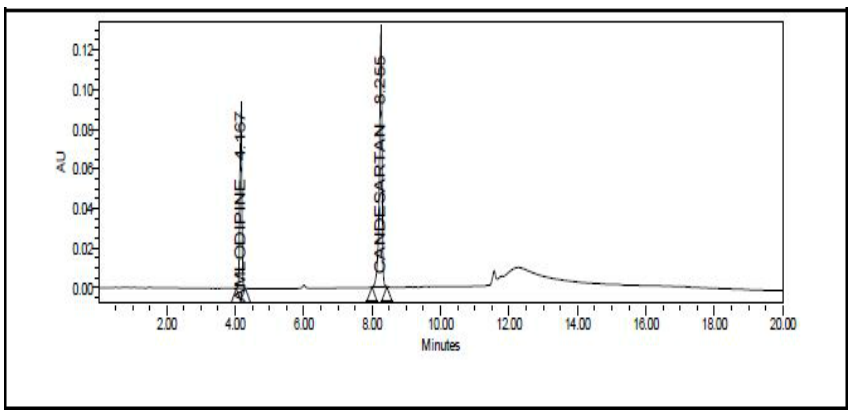


Fig. no. 9a: Chromatogram of photolytic degerdation (Open).

Table no. 10a: Result and observation for Photolytic degradation (Open).

Sample	Peak area (Uv)	% assay	% degradation	Purity angle	Purity threshold	Flag
AMLO	356967	98.66	0.54	0.706	1.132	NO
CANDE	679457	98.76	0.67	0.533	1.122	NO

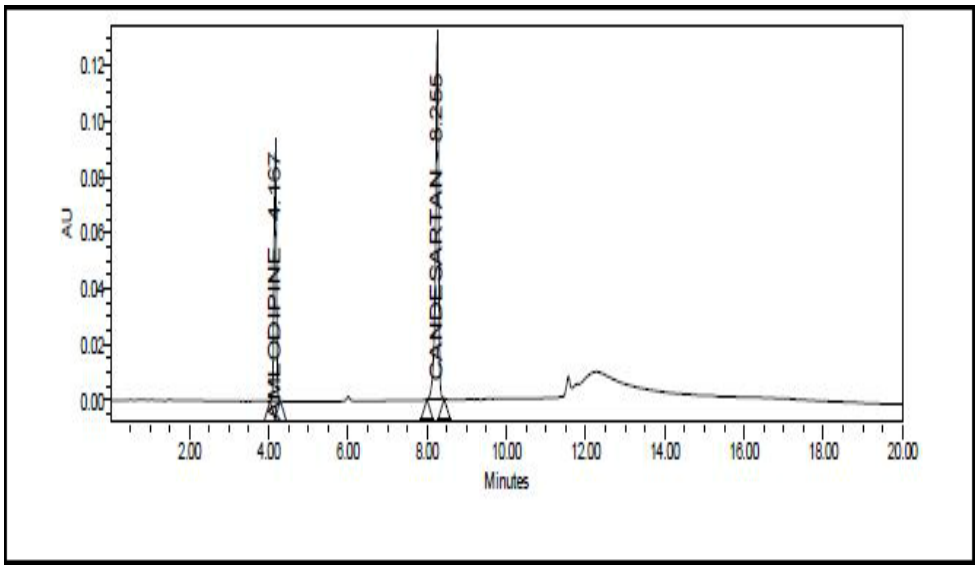


Fig. no. 9b: Chromatogram of photolytic degerdation (Close).

Table No. 10b: Result and observation for photolytic degratation (Close).

Sample	Peck area(uV)	% Assay	% Degradation	Purity Angle	Purity Threshold	Flag
AMLO	326967	91.44	7.82	0.706	1.223	NO
CANDE	690185	99.61	-	0.533	1.122	NO

Validation of proposed method

The developed method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. Validation was done as per ICH guidelines Q2 (R1). The developed method was validated with respect to parameters such as linearity, Limit of detection (LOD) and LOQ, precision, accuracy and specificity

Specificity

Chromatogram for the blank, placebo and standard to determine their any peck in blank and placebo which may interfere with standard peak.

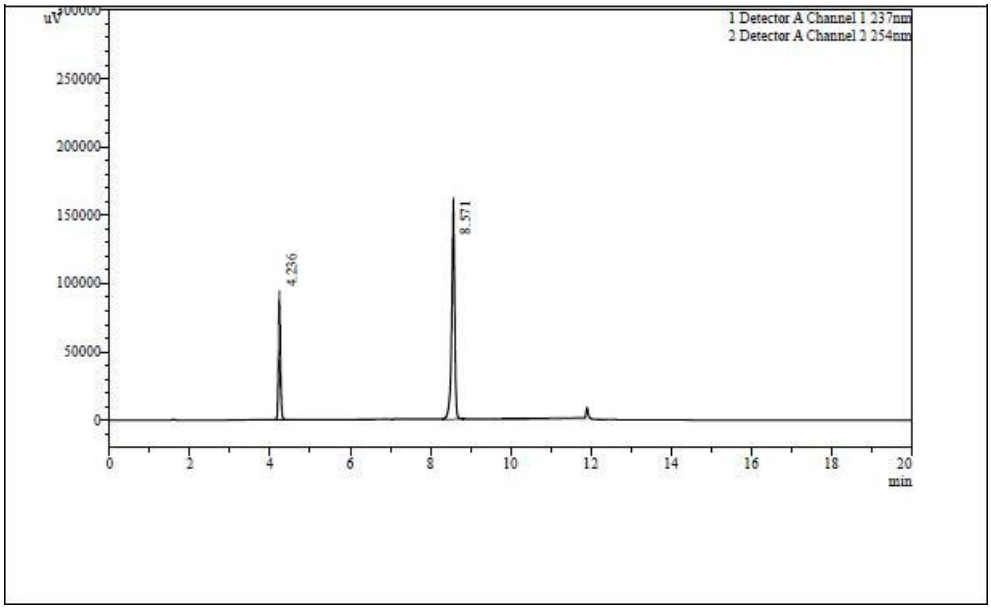


Fig. no. 10: Chromatogram for standard solution.

Linearity and Range

The linearity of an analytical procedure is its ability (within given range) to obtained test results which are the directly proportional to the concentration (amount) of analyte in sample.The linearity of detector response for drug with respect to concentration was demonstrated by considering 100% target concentration [28 ug/ml] of Amlodipine Besylate and [64 ug /ml] of Candesartan cilexetil and preparing solution in diluent with concentration ranging from about 50 % to 150 % of the target concentration and the graph was plotted with concentration on X-axis and mean peck area on Y- axis. Which was reported at Table No.6.1.4 and Fig.No.6.1.4a and b.

Accuracy

Accuracy of the proposed method was ascertained on the basis of recovery studies performed. Result are shown in Table No. 4a and 4b.

Precision

The precision of an analytical procedure express the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple sample of the same homogeneous sample under the prescribed condition. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The precision of an analytical procedure is usually expressed as the variance, standard deviation, relative standard deviation or coefficient of variation of a series of measurements.

System precision

Procedure

Precision of any analytical method is expressed as SD and RSD of series of measurement. System precision was ascertained by replicate injecting 20ul of each standard solution and chromatogram were recorded. Result are shown in Table No.11

Table no. 11: Observation and result of system precision.

Injection	AUC of AMLO (uV)	AUC of CANDE
1	373269	715717
2	373475	716420
3	373418	716569
4	373310	716295
5	373603	717050
6	373453	717888
MEAN	373421.3333	716656.5
SD	120.4137	741.3994
%RSD	0.03	0.10

Method precision

Procedure: Method precision was ascertained by replicate injecting 20ul of each sample solution and chromatogram were recorded. Result are shown in Table No.12

Table no. 12: Observation and result of method precision.

Injection	%Label Claim of AMLO		% Label Claim of CANDE
1		100.5	99.2
2		101.2	99.9
3		100.9	99.4
4		101.1	99.9
5		101.5	100.3
6		100.7	99.5
MEAN		100.98	99.7
SD		0.36	0.40
%RSD		0.36 %	0.41%

Ruggedness

It expresses within laboratory variation. This attribute evaluates the reliability of the method in an environment different from that used during the method development phase.

Interday and Intraday

The mix sample was prepared as per the procedure described under bulk formulation and analyzed at intervals of 0hr., 3hr., 5hr., for interday study and on 1st and 3rd for intraday

study. The content of AMLO and CANDE was calculated by formula and the results obtained for intraday and intraday study. The result are shown in Table No.13 and 14

Table no. 13: Observation and Result estimation in intraday.

Time	AUC (uV)		% Label Claim	
	AMLO	CANDE	AMLO	CANDE
0 hr.	369702	720441	100.2	99.9
3hr.	372553	720497	101.2	99.9
5hr.	371549	717041	100.9	99.4
	Mean		100.7	99.7
	S.D.		0.3674	0.2061
	%RSD		0.0036%	0.00206%

Table no. 14: Observation and Result estimation in interday.

Time	AUC (uV)		% Label Claim	
	AMLO	CANDE	AMLO	CANDE
1Day	369702	720441	100.2	99.9
3Day	358054	675111	99.2	98.1
	Mean		99.7	99
	S.D.		0.7071	1.2727
	%RSD		0.00709%	1.29%

Different analyst

The sample was prepared and analyzed as per the proposed method. The ruggedness of the proposed method has been verified by analyzing as triplicate of tablet sample used for the method precision by two different analysts using same instrument. The ruggedness result were compared with method precision data. The overall mean, standard deviation (SD) and %RSD of the assay value are shown in Table No. 15

Table No. 15: Observation and Result estimation in different analysis.

Sr. No.	% Estimation of AMLO		% Estimation of CANDE	
	Analysist-I	Analysist-II	Analysist-I	Analysist-II
1	100.5	99.8	99.2	100.1
2	101.5	99.4	99.9	100.8
3	100.9	100.8	99.4	100.4
Mean	100.96	100.0	99.5	100.43
SD	0.5032	0.7211	0.36055	0.3511
%RSD	0.50%	0.72 %	0.36%	0.35%

Robustness

The robustness of the method was evaluate by deliberately varying the chromatographic condition viz. flow rate by ±0.2 ml, column over temperature by ±5° C. at these changed

condition the standard and sample solutions were injected. The system suitability was evaluated in each varied condition. The amount of AMLO and CANDE was calculated from sample solution in each varied condition. The results were shown in Table No. 16 and 17.

I	Flow rate (1.3ml/min)
II	Flow rate (1.7ml/min)
III	Column oven temperature (45°C)
IV	Column oven temperature(35°C)

Table no. 16: Observation and result of robustness for amlodipine besylate.

Sr. No.	I (%)	II (%)	III (%)	IV (%)
1	101.19	98.92	99.79	98.72
2	101.07	99.02	99.82	98.73
3	102.02	98.85	100.16	99.15
4	100.89	98.75	99.97	98.90
5	101.26	98.67	99.90	99.69
6	101.16	98.99	99.91	98.73
Over all mean	101.26	98.30	99.92	98.97
Over all SD	0.3912	0.8000	0.1321	0.3978
Over all %RSD	0.39 %	0.81 %	0.13 %	0.40 %

Table no. 17: Observation and result of robustness for candesartan cilexetil.

Sr. No.	I (%)	II (%)	III (%)	IV (%)
1.	101.05	98.04	100.01	100.22
2.	100.49	99.31	99.82	99.91
3.	100.28	99.20	99.89	100.42
4.	101.09	99.09	100.08	100.09
5.	102.24	98.42	99.91	99.09
6.	102.01	98.02	100.48	99.10
Over all mean	101.19	98.66	100.03	99.80
Over all SD	0.7902	0.5822	0.23	0.57
Over all %RSD	0.78 %	0.59 %	0.24 %	0.58 %

RESULT AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Amlodipine Besylate and Candesartan Cilexetil preferably analyzed by reverse phase columns and accordingly C18 column was selected so the elution of the compound from the column was influenced by polar mobile phase. The concentration of the Ortho Phosphoric Acid and Acetonitrile were optimized to give symmetric peaks with short run time based on asymmetric factor and peak are obtained. Different mobile phase tried but Acetonitrile: Water: Ortho Phosphoric Acid (950:50:01) and Acetonitrile: Water:

Ortho Phosphoric Acid (50:950:01) gives well resolved and good symmetrical peaks. The retention time for Amlodipine Besylate and Candesartan Cilexetil were found to be 4.236 min and 8.571 min minute respectively. The RSD value for accuracy and precision is less than 2% which indicate that developed method is accurate and precise. The degree of reproducibility of the result obtained as a result of small and deliberate variations in the method parameter has proven that the method is robust. The results of assay indicate that the method is selective for the analysis of synthetic mixture of drug Amlodipine Besylate and Candesartan Cilexetil.

CONCLUSION

The results obtained by proposed HPLC method for simultaneous estimation of Amlodipine Besylate and Candesartan Cilexetil in dosage form was found to be reliable, precise, and accurate. Also proposed validated HPLC method indicates stability of drugs under stress conditions. Hence it can be routinely adopted for analysis of dosage form.

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