

ESTIMATION OF AMLODIPINE BESYLATE AND IRBESARTAN IN PHARMACEUTICAL FORMULATION BY RP-HPLC WITH FORCED DEGRADATION STUDIES

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ABSTRACT

Objective: To develop and validate a simple, specific, accurate, precise and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method with forced degradation studies for the simultaneous estimation of amlodipine besylate and irbesartan in the pharmaceutical formulation.

Methods: The chromatographic separation of the two drugs were achieved using Enable C 18G column (250 × 4.6 mm; 5 μm) in isocratic mode with mobile phase consisting of sodium acetate buffer (pH 4.0) and acetonitrile (30:70, % v/v) with a flow rate of 0.6 ml/min. Ultraviolet(UV) detection was carried out at 238 nm. The proposed method was validated for linearity, range, accuracy, precision, robustness, limit of detection (LOD) and limit of quantification (LOQ). The tablet formulation was subjected to stress conditions of degradation including acidic, alkaline, oxidative, thermal and photolysis.

Results: The retention time for amlodipine besylate and irbesartan were found to be 5.512 and 6.321 min respectively. Linearity was observed over a concentration range 4-32 μg/ml for amlodipine besylate ($r^2 = 0.9999$) and 10-70 μg/ml for Irbesartan ($r^2 = 0.9998$). The % relative standard deviation (RSD) for Intraday and Interday precision was found to be 0.436 and 0.699 for amlodipine besylate and 0.435 and 0.30 for irbesartan. Amlodipine besylate shown stability towards acidic and thermal whereas in basic, oxidative and photolytic it shown less stability in which it degraded to more extent. Irbesartan shown stability towards thermal conditions whereas in remaining conditions it degrades to more extent especially in oxidative conditions.

Conclusion: The developed reverse phase high performance liquid chromatographic (RP-HPLC) method was also found to be simple, precise and sensitive for the simultaneous determination of amlodipine besylate and irbesartan in the tablet dosage form.

Keywords: Amlodipine Besylate, Irbesartan, RP-HPLC and Forced Degradation

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INTRODUCTION

Amlodipine besylate (fig. 1), chemically designated as 2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl) 1,4-dihydro-6-methyl-3,5-pyridine-dicarboxylic acid-3 ethyl-5 methyl ester, is a calcium channel blocker used to treat hypertension and angina. The drug is found to metabolize in the liver and the produced metabolites are excreted via urine along with some unchanged drug. Literature survey reveal various analytical method are reported either alone or combination with other drugs includes UV spectrophotometric[1-4], Visible spectrophotometric [5-7], spectrofluorometric [8], Titrimetry [5], LC-MS [9], HPLC [10, 11] in pure drug, pharmaceutical formulations and biological fluids.

Irbesartan (fig. 2) is an angiotensin II receptor antagonist used in the management of hypertension including treatment of renal disease in hypertensive type II diabetic patients. It possesses an acidic tetrazole system and biphenyl system, does not have acidic side chain and even so it has good affinity for angiotensin II receptor because of hydrogen bonding with the carbonyl moiety of amide system. It decreases afterload and preload. It is more effective in providing 24 hr control of blood pressure. Combination therapy of hypertension with different categories offers the potential to lower blood pressure more quickly, obtain target blood pressure, and decrease adverse effects. The combination tablet containing amlodipine 10 mg and irbesartan 100 mg had a greater BP-lowering effect than an angiotensin receptor blocker (ARB) and a low-dose or regular-dose calcium channel blocker (CCB). In addition, the combination tablet had more favorable effects on serum uric acid, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol levels in patients with hypertension [12]. Several analytical methods have been reported for the determination of Irbesartan either alone or in combination with other drugs in pure drug, pharmaceutical dosage forms and in biological samples using spectrophotometry [13-16], HPLC [17-39], HPTLC [40-42], LC-MS [43-45], and voltammetry [46] for the determination of irbesartan in

pharmaceutical dosage forms. The combination of amlodipine besylate and irbesartan is effective in the treatment of hypertension. Various analytical methods were reported for simultaneous estimation of amlodipine besylate and irbesartan in pure drug, pharmaceutical formulations and biological fluids by HPLC [47-49]. Only one stability indicating RP-HPLC method was reported for the simultaneous estimation of both drugs in pharmaceutical formulation but the developed method has long retention time and low linearity range for both drugs. Therefore in the present study, an attempt was made to develop a simple, precise, accurate RP-HPLC method with forced degradation studies for the analysis of amlodipine besylate and irbesartan in pharmaceutical formulation.

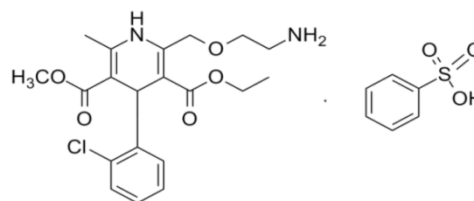


Fig. 1: Structure of amlodipine besylate

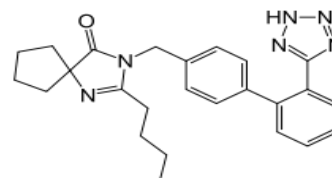


Fig. 2: Structure of Irbesartan

MATERIALS AND METHODS

Materials and chemicals

Amlodipine Besylate and Irbesartan standard were obtained as gifted sample from pharma industry. Amlodipine Besylate and Irbesartan Tablets (AMIX R TABLETS) containing Irbesartan 100 mg and amlodipine 10 mg were purchased from a local pharmacy. HPLC grade Acetonitrile and water were from MERCK India Ltd. HPLC grade methanol was from standard reagent pvt ltd Hyderabad. Analytical grade acetic acid, sodium acetate, hydrochloric acid, sodium hydroxide and hydrogen peroxide were from SD Fine chemicals Mumbai, India. Nylon membrane filters 0.2 μm and 0.45 μm were from PALL life sciences Mumbai India. Ultrasonicator used was from LAB india Ltd Mumbai. pH meter was of Elico LI 120 make. UV spectrophotometer was of Elico SL 210 model consisted of spectral treats software.

Instrumentation

The chromatographic system used for the method development and validation consisted of Shimadzu HPLC comprising of LC-20AD binary gradient pump, a variable wavelength programmable SPD-20A detector and an SCL 20A system controller. A Rheodyne injector 7725i fitted with a 20 μL loop was used and data were recorded and evaluated by use of LC solutions software version 5.0.

Chromatographic conditions

Chromatographic analysis was performed on Enable C18 G column (250 x 4.6 mm i. d, 5 μ). The mobile phase consisted of sodium acetate buffer (pH 4.0) and acetonitrile (30:70, %v/v). The flow rate was 0.6 ml/min, injection volume was 20 μl and detection was carried out at 238 nm using a UV detector.

Preparations of amlodipine besylate and irbesartan stock solution

Stock solution of amlodipine besylate (1000 $\mu\text{g/ml}$) and irbesartan(1000 $\mu\text{g/ml}$) was prepared separately by transferring accurately weighed 50 mg of amlodipine besylate and 50 mg of Irbesartan into a 50 ml volumetric flask and to it added a 20 ml methanol. The mixture was sonicated for 5 min to dissolve the drug and the solution was diluted up to the mark with methanol. Standard solution amlodipine besylate (100 $\mu\text{g/ml}$) and irbesartan (100 $\mu\text{g/ml}$) were prepared by diluting 10 ml of standard stock solution to 100 ml in a volumetric flask with mobile phase. To prepare a binary mixture of irbesartan and amlodipine besylate appropriate volume of standard solution was transferred into a 100 ml volumetric flask and diluted with mobile phase to get a solution containing 100 $\mu\text{g/ml}$ of irbesartan and 10 $\mu\text{g/ml}$ of amlodipine besylate.

Analysis of amlodipine besylate and irbesartan in combined dosage form

Accurately weighed about twenty tablets and average weight of the tablet was determined. The tablets were transferred into mortar and triturated to a fine powder form. An aliquate of the powder equivalent to 100 mg of irbesartan and 10 mg of amlodipine besylate was transferred into a 100 ml volumetric flask. To it 20 ml HPLC grade methanol was added and sonicated for 5 min to dissolve the drugs. The content of the flask was kept for 10 min at laboratory temperature and diluted up to mark with HPLC grade methanol this gives a concentration of irbesartan 1000 $\mu\text{g/ml}$ and amlodipine besylate 100 $\mu\text{g/ml}$. The above solution was filtered through 0.2 μ membrane filter. The 6 ml of the filtrate was transferred into a 100 ml volumetric flask and diluted with mobile phase to get a concentration of 60 $\mu\text{g/ml}$ and 6 $\mu\text{g/ml}$ for irbesartan and amlodipine besylate respectively.

Method validation

The method was validated for accuracy, precision, linearity, specificity, robustness, limit of detection, limit of quantitation and system suitability.

Linearity

Linearity was performed by preparing standard solutions of irbesartan and amlodipine besylate at different concentration levels.

Irbesartan was prepared in the concentration range of 10-70 $\mu\text{g/ml}$ and 4-32 $\mu\text{g/ml}$ for amlodipine besylate. Twenty microlitres of each concentration from both drug solutions were injected in duplicate into the HPLC system. The response was carried out at 238 nm and the corresponding chromatograms were recorded from these mean peak areas were calculated. The calibration curve was plotted by taking concentration on x-axis and peak areas on y-axis for both the drugs.

Accuracy

The accuracy of the method evaluated by standard addition method in which a known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery of irbesartan and amlodipine besylate was calculated at three concentration levels of 80 %, 100 % and 120 %. The solutions were analyzed in triplicate at each level. The percent recovery and % relative standard deviation (RSD) at each level was calculated.

Precision

Precision of the method was evaluated as system precision and method precision.

To study the system precision, six replicate standard solutions of irbesartan and amlodipine besylate were analysed. The percent relative standard deviation (% RSD) was calculated for both irbesartan and amlodipine besylate.

Method precision of the analytical method was carried out on six preparations from the tablet formulation and percentage amount of irbesartan and amlodipine besylate in the tablet formulation was calculated. The intraday and interday precision study were conducted for both irbesartan and amlodipine besylate. The mean % assay value, standard deviation and percent relative standard deviation was calculated [50].

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD was measured by serially diluting the standard solutions of irbesartan and amlodipine besylate and determining the concentration was the response of sample peaks are three times the noise peak. LOQ was measured by serially diluting the standard solutions of irbesartan and amlodipine besylate and determining the concentration was the response of sample peaks are ten times the noise peak [51].

Robustness

Robustness of the method was determined by making slight changes in the composition of organic phase $\pm 5\%$, pH ± 0.2 , flow rate ± 0.1 ml/min and detection wavelength ± 2 nm.

Specificity

The specificity of the proposed method was determined against blank and placebo applications. Here mobile phase was used as blank and excipients like starch, lactose, magnesium stearate were used as placebo [52].

Forced degradation studies

Different stress conditions were used for the forced degradation studies of the formulation. These were also used to evaluate the specificity of the method. All the samples were diluted with mobile phase and filtered through 0.2 μ membrane filter.

Acidic conditions

Weighed accurately about twenty tablets and triturated it to a fine powder form. An aliquate of the powder equivalent to 100 mg of irbesartan and 10 mg of amlodipine besylate was transferred into a 100 ml volumetric flask. To this added a 50 ml of diluent and sonicated for 10 min to dissolve the drug completely. Then 10 ml of 5N HCl was added to it, refluxed for 6 hr at 60 $^{\circ}\text{C}$, cooled to room temperature, neutralized with 5N NaOH and diluted up to the mark with the diluent. The above sample solution was filtered through 0.2 μ nylon membrane filter. Pipetted 6 ml of the above filtered sample solution into a 100 ml volumetric flask and volume made up to the mark with diluent.

Alkaline conditions

Weighed accurately about twenty tablets and triturated it to a fine powder form. An aliquate of the powder equivalent to 100 mg of irbesartan and 10 mg of amlodipine besylate was transferred into a 100 ml volumetric flask. To this added a 50 ml of diluent and sonicated for 10 min to dissolve the drug completely. Then 10 ml of 5N NaOH was added to it, refluxed for 6 hr at 60 °C, cooled to room temperature, neutralized with 5N HCl and diluted up to the mark with the diluent. The above sample solution was filtered through 0.2 µ nylon membrane filter. Pipetted 6 ml of the above-filtered sample solution into a 100 ml volumetric flask and volume made up to the mark with diluent.

Oxidative degradation

Weighed accurately about twenty tablets and triturated it to a fine powder form. An aliquate of the powder equivalent to 100 mg of irbesartan and 10 mg of amlodipine besylate was transferred into a 100 ml volumetric flask. To this added a 50 ml of diluent and sonicated for 10 min to dissolve the drug completely. Then 5 ml of 30 % hydrogen peroxide was added, refluxed for 2 hr at 60 °C, then cooled to room temperature and diluted up to the mark with diluents. The above sample solution was filtered through 0.2 µ nylon membrane filter. Pipetted 6 ml of the above-filtered sample solution into a 100 ml volumetric flask and volume made up to the mark with diluent.

Thermal degradation

Weighed accurately about twenty tablets and triturated it to a fine powder form. The powder sample was subjected to thermal stress at 105 °C for about 2 d. An aliquate of the powder equivalent to 100 mg of irbesartan and 10 mg of amlodipine besylate was transferred into a 100 ml volumetric flask. To this added a 50 ml of diluent and sonicated for 10 min to dissolve the drug completely the diluted up to mark with diluents. The above sample solution was filtered through 0.2 µ nylon membrane filter. Pipetted 6 ml of the above-filtered sample solution into a 100 ml volumetric flask and volume made up to the mark with diluent.

Photolytic degradation

Weighed accurately about twenty tablets and triturated it to a fine powder form. The powder sample was subjected to UV light in a photo stability chamber for about 10 d. An aliquate of the powder

equivalent to 100 mg of irbesartan and 10 mg of amlodipine besylate was transferred into a 100 ml volumetric flask. To this added a 50 ml of diluent and sonicated for 10 min to dissolve the drug completely the diluted up to mark with diluents. The above sample solution was filtered through 0.2 µ nylon membrane filter. Pipetted 6 ml of the above-filtered sample solution into a 100 ml volumetric flask and volume made up to the mark with diluent.

RESULTS

Optimization of chromatographic conditions

In the present work, an analytical method based on RP-HPLC using UV detection was developed and validated for simultaneous estimation of irbesartan and amlodipine besylate in the pharmaceutical formulation. The selection of analytical conditions was based on the chemical nature of irbesartan and amlodipine besylate. A systematic study of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant for development of an analytical method. Both irbesartan and amlodipine besylate were soluble in polar solvents, therefore, RP-HPLC was chosen. The selection of the stationary phase has been done on the basis of back pressure, resolution, peak shape, theoretical plates and day to day reproducibility in retention time resolution between irbesartan and amlodipine besylate peaks. After evaluating all these factors Enable C18 G column (250 x 4.6 mm i. d, 5µ) was chosen for the analysis. The selection of buffer was based on the chemical structure of selected drug molecules. For optimization of mobile phase, preliminary trials were conducted under isocratic conditions using mobile phases composed of the mixture of solvents like water, methanol and acetonitrile with or without different buffers in different combinations. A mixture of sodium acetate buffer pH 4.0 and acetonitrile in the ratio of 30:70 v/v was found to be most suitable of all the combinations since the chromatographic peaks obtained were having good system suitability parameters. The Flow rate of the mobile phase was optimized based on the resolution between chromatographic peaks and minimal solvent consumption. The flow rate of mobile phase was changed from 0.5-2 ml/min. It was found from trials that 0.6 ml/min flow rate was ideal for successful elution of both drugs. For a selection of analytical wavelength standard solutions of both drugs were scanned in the wavelength range of 200-350 nm. A detection wavelength of 238 nm was selected. The chromatogram of the sample was shown in fig. 3.

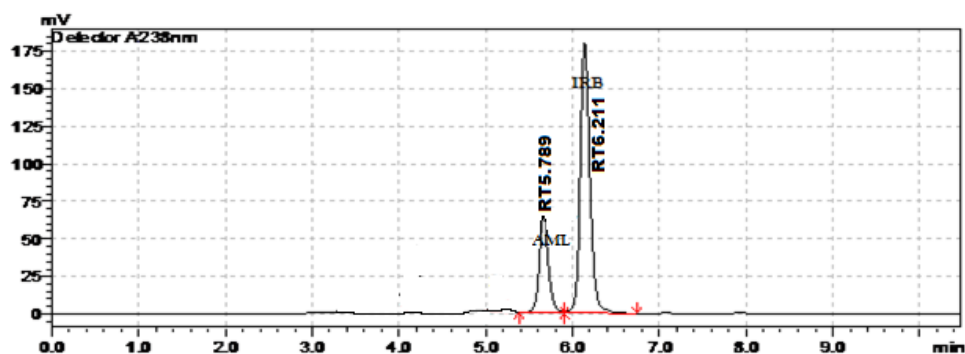


Fig. 3: Chromatogram of amlodipine besylate and Irbesartan

Table 1: Linearity data for amlodipine besylate

S. No.	Concentration of amlodipine besylate(µg/ml)	Peak area
1	4	191223
2	8	382459
3	12	573701
4	16	764877
5	20	956106
6	24	1147398
7	28	1338561
8	32	1529772
Slope		47806
Intercept		11.786
Correlation Coefficient		0.9999

Method validation

Linearity was studied by preparing standard solutions at different concentration levels. The linearity ranges for amlodipine besylate and irbesartan were found to be 4-32 µg/ml and 10-70 µg/ml respectively. The linear regression equation for amlodipine besylate was found to be $47806x+11.786$ with correlation coefficient 0.9999. The linear regression equation for irbesartan was found to be $62825x-16.143$ with correlation coefficient 0.9998. The calibration table

for amlodipine besylate and irbesartan was shown in table 1 and table 2 respectively. The calibration curve of amlodipine besylate and irbesartan were shown in fig. 4 and fig. 5 respectively.

Accuracy

The percent recovery of irbesartan and amlodipine besylate was found to be 100.43-101 % and 100.07-100.52 %. This indicates the accuracy of the method. The results are shown in table 3 and 4.

Table 2: Linearity data for Irbesartan

S. No.	Concentration of Irbesartan(µg/ml)	Peak area
1	10	628247
2	20	1256484
3	30	1884758
4	40	2512971
5	50	3141220
6	60	3769498
7	70	4397771
Slope		62825
Intercept		-16.143
Correlation Coefficient		0.9998

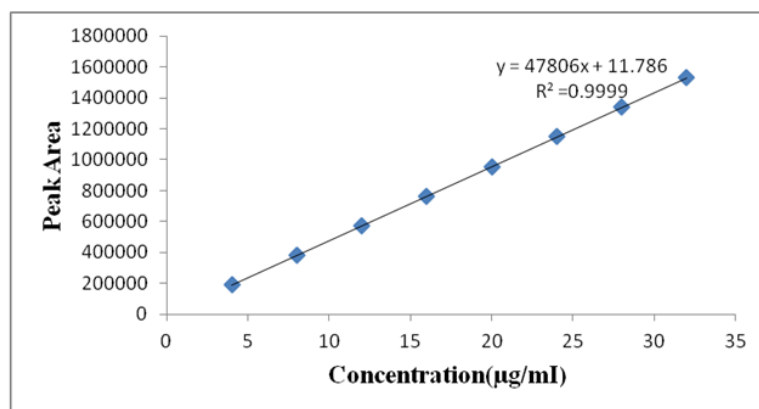


Fig. 4: Linearity plot of amlodipine besylate

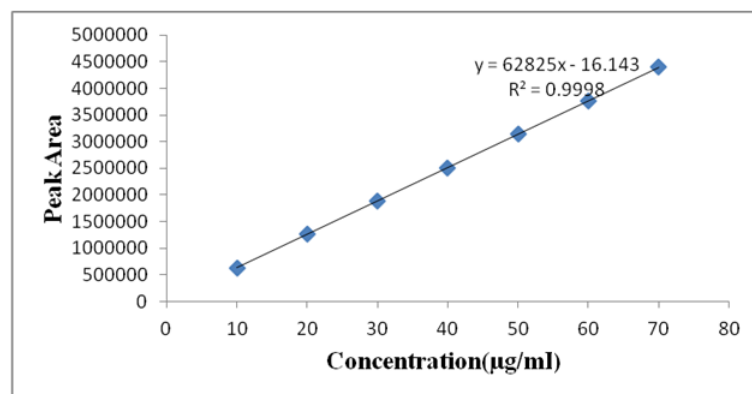


Fig. 5: Linearity plot of irbesartan

Table 3: Accuracy results of Irbesartan (n=3)

Drug Name	Level of addition (%)	Amount taken (µg/ml)	Amount found (µg/ml (mean±SD))	% recovery (mean±SD)
Irbesartan	80	48	48.41	100.88±0.80
	100	60	60.25	100.43±0.31
	120	72	72.71	101±0.75

n is number of determinations, SD is standard deviation

Table 4: Accuracy results of amlodipine besylate (n=3)

Drug Name	Level of addition (%)	Amount taken ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$ (mean \pm SD))	% recovery (mean \pm SD)
Amlodipine Besylate	80	4.8	4.80	100.07 \pm 0.41
	100	6.0	6.02	100.52 \pm 0.21
	120	7.2	7.22	100.45 \pm 0.51

n is number of determinations, SD is standard deviation

Table 5: System precision results for irbesartan and amlodipine besylate

Injection No.	Peak area of irbesartan	Peak area of amlodipine besylate
1	3769498	286847
2	3713456	289114
3	3728862	279912
4	3719841	285318
5	3758423	284877
6	3788912	286006
Mean	3746499	285345.7
SD	30277.22	3054.03
%RSD	0.80	1.07

Table 6: Method precision results for Irbesartan and amlodipine besylate (n=6)

Set	Irbesartan		Amlodipine besylate	
	Intraday (mean \pm SD)	Interday (mean \pm SD)	Intraday (mean \pm SD)	Interday (mean \pm SD)
% Assay	100.69 \pm 0.4385	101.04 \pm 0.3033	99.76 \pm 0.435	100.13 \pm 0.704
% RSD	0.435	0.30	0.436	0.699

n is number of determinations, SD is standard deviation, RSD is relative standard deviation

Precision

System precision

The % relative standard deviation (RSD) for irbesartan was found to be 0.80 and for amlodipine besylate was found to be 1.07 which are within the acceptance criteria of not more than 2.0 indicates the precision of the method. The precision data was shown in table 5.

Method precision

The % relative standard deviation (RSD) for Intraday and Interday precision assay results of six preparations for irbesartan were found to be 0.435 and 0.30 respectively which are within the acceptance criteria of not more than 2.0 indicates the precision of the method. The % relative standard deviation (RSD) for Intraday and Interday precision assay results of six preparations for amlodipine besylate were found to be 0.436 and 0.699S respectively which are within the acceptance criteria of not more than 2.0 indicates the precision of the method. The precision data was shown in table 6.

Limit of detection and limit of quantitation

LOD and LOQ were calculated from the standard deviation of response and slope of the calibration curve of irbesartan and amlodipine besylate. The LOD and LOQ were found to be 0.0327 $\mu\text{g/ml}$ and 0.1097 $\mu\text{g/ml}$ for amlodipine besylate and the LOD and LOQ for irbesartan were 0.0514 $\mu\text{g/ml}$ and 0.1559 $\mu\text{g/ml}$, which

shown that the developed method was sensitive and it can detect and quantify at the lower concentration.

Robustness

To evaluate the robustness of the developed method, small deliberate variations in optimized method parameters were made. The effect of the change in flow rate, change in pH, change in the composition of mobile phase and detection wavelength on retention time, tailing factor and theoretical plates were studied. The method was found to be unaffected by small changes in flow rate, change in pH, change in the composition of mobile phase and detection wavelength as shown in table 7 and table 8.

Specificity

Specificity is the ability to unequivocally assess the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degradants or matrix. Specificity of an analytical method is its ability to accurately and specifically measure the analyte of interest without interference from blank or placebo. The peak purities of amlodipine besylate and irbesartan were assessed by comparing the retention times of standard amlodipine besylate and irbesartan and the sample, and good correlation was obtained between the retention time of the standard and sample. Placebo and blank were injected and there were no peaks. There is no interference of degradation peaks on drug peaks hence, the method is specific. The specificity results are shown in table 9.

Table 7: Robustness results for amlodipine besylate (n=3)

Conditions	% Assay (mean \pm SD)	System suitability parameters	
		Theoretical plates (mean \pm SD)	Tailing factor (mean \pm SD)
Flow Rate 0.5 ml/min	99.23 \pm 0.33	5309 \pm 0.37	1.21 \pm 0.39
Flow Rate 0.7 ml/min	99.14 \pm 0.21	5105 \pm 0.67	1.49 \pm 0.31
Mobile Phase-Buffer(35):Acetonitrile(65)	100.83 \pm 0.78	4983 \pm 0.42	1.42 \pm 0.89
Mobile Phase-Buffer(25):Acetonitrile(75)	99.12 \pm 0.96	4862 \pm 0.28	1.39 \pm 0.88
Mobile Phase p ^H 3.8	101.32 \pm 0.66	4821 \pm 0.91	1.61 \pm 0.60
Mobile Phase p ^H 4.2	99.55 \pm 0.54	5129 \pm 0.56	1.59 \pm 0.95
Wavelength 236 nm	99.68 \pm 0.85	5157 \pm 0.44	1.53 \pm 0.87
Wavelength 240 nm	99.87 \pm 0.21	5004 \pm 0.36	1.52 \pm 0.92

n is number of determinations, SD is the standard deviation

Table 8: Robustness results for Irbesartan (n=3)

Conditions	% Assay (mean±SD)	System suitability parameters	
		Theoretical plates (mean±SD)	Tailing factor(mean±SD)
Flow Rate 0.5 ml/min	99.82±0.48	3340±0.67	1.33±0.87
Flow Rate 0.7 ml/min	99.74±0.66	3030±0.39	1.54±0.91
MobilePhase-Buffer(35):Acetonitrile(65)	100.25±0.75	3289±0.74	1.23±0.48
Mobile Phase-Buffer(25):Acetonitrile (75)	100.63±0.58	3373±0.84	1.28±0.87
Mobile Phase p ^H 3.8	99.66±0.67	3116±0.61	1.53±0.37
Mobile Phase p ^H 4.2	100.79±0.91	3193±0.59	1.34±0.54
Wavelength 236 nm	99.83±0.49	3288±0.78	1.37±0.82
Wavelength 240 nm	99.78±0.78	3462±0.42	1.35±0.69

n is number of determinations, SD is standard deviation

Table 9: Specificity results of the method (n=6)

Name of solution	Retention time (min) (mean±SD)
Blank	No peaks
Placebo	No Peaks
Irbesartan	6.321±0.78
Amlodipine Besylate	5.579±0.91

n is number of determinations, SD is standard deviation

Table 10: Analysis of amlodipine besylate and Irbesartan in the commercial formulation

Formulation	Labelled claim(mg)		Amount found*(mg)		%Recovery*±%RSD	
	Amlodipine besylate	Irbesartan	Amlodipine besylate	Irbesartan	Amlodipine besylate	Irbesartan
AMIX R TABLETS	10	100	9.96	100.06	99.96±0.42	100.6±0.56

*Average of three determinations

Analysis of commercial formulation

The proposed method was applied for the determination of amlodipine besylate and irbesartan in marketed formulations available (AMIX R TABLETS). The % recovery was found to be 99.96±0.42 and 100.6±0.56 for amlodipine besylate and irbesartan respectively table 10.

Results of forced degradation studies

Under acidic conditions amlodipine, besylate degraded to 3.44 % and irbesartan degraded to 10.66 %. In these stress conditions, the retention time of degradation peaks appears at 4.2132 min and 5.182 min. In basic conditions amlodipine, besylate degraded to 30.44 % and irbesartan degraded to 18.46%. Under these conditions, five degradant peaks appear at retention times of 3.812 min, 4.252 min, 4.754 min, 5.255 min and 9.511 min. In oxidative

conditions amlodipine, besylate degraded to 30.36 % and irbesartan to 30.42 %. Although both drugs degraded to a significant extent but only two minor peaks are detected at retention times of 3.345 min and 4.366 min. In thermal conditions amlodipine, besylate degraded to 0.43 % and irbesartan degraded to 5.06 % but there is no appearance of degradant peaks on the chromatogram. In photolytic conditions amlodipine, besylate degraded to 28.72 % and irbesartan degraded to 15.66 % here also both the drugs degraded to a significant extent but only two minor peaks are detected at retention times of 3.332 min and 4.414 min. Amlodipine besylate shown stability towards acidic and thermal in it degraded to a lesser extent whereas in basic, oxidative and photolytic it shown less stability in which it degraded to more extent. Irbesartan shown stability towards thermal conditions whereas in remaining conditions it degrades to more extent especially in Oxidative conditions.

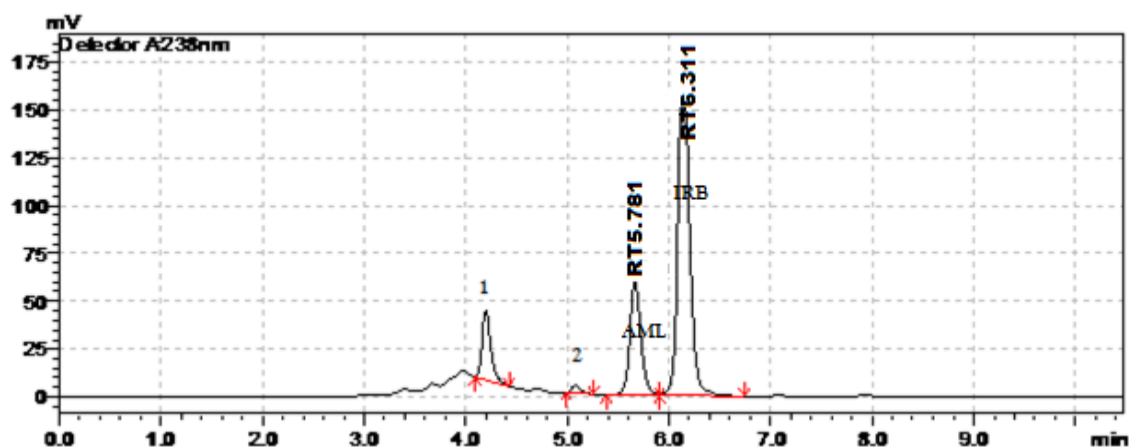


Fig. 6: Acid degradation chromatogram

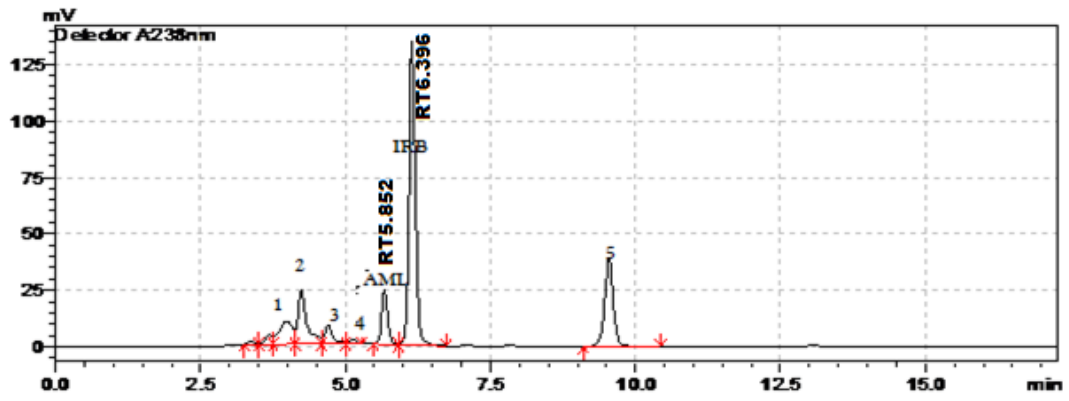


Fig. 7: Base degradation chromatogram

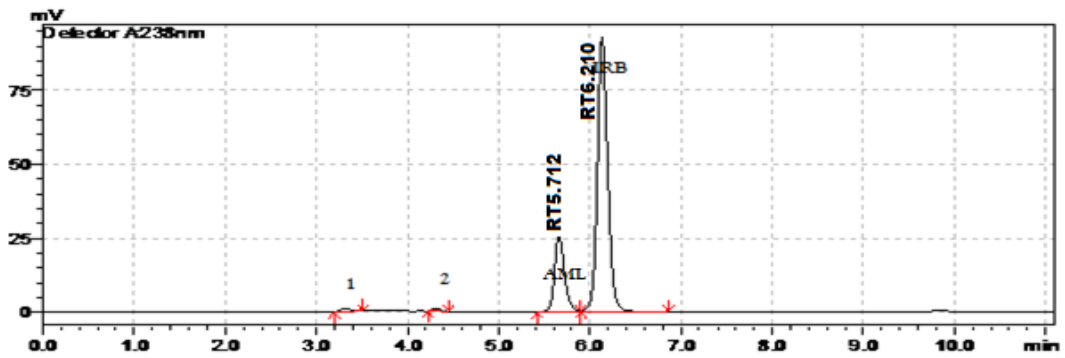


Fig. 8: Oxidative degradation chromatogram

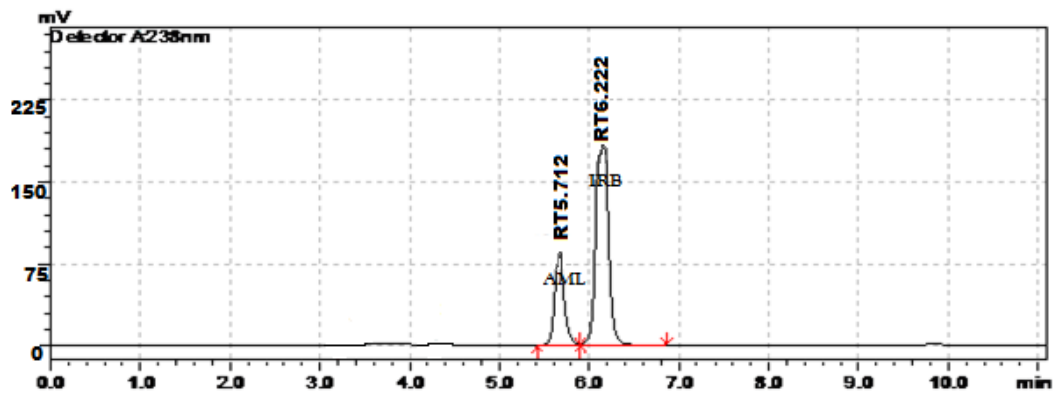


Fig. 9: Thermal degradation chromatogram

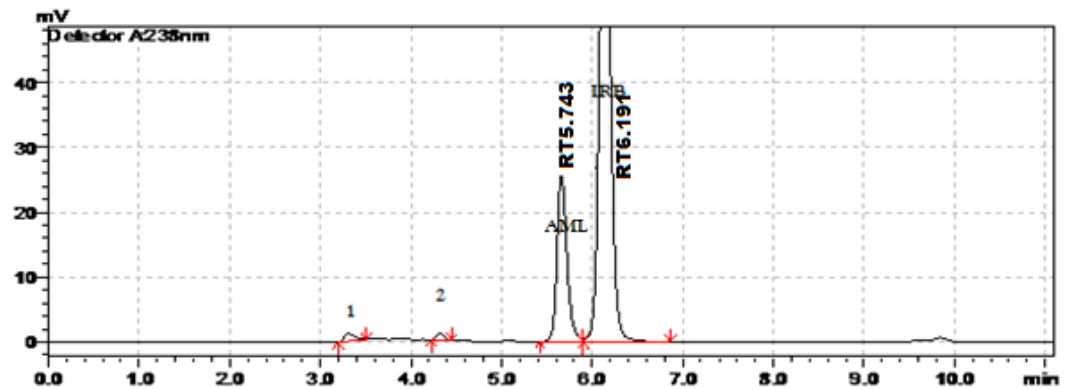


Fig. 10: Photolytic degradation chromatogram

Table 11: Forced degradation studies of Irbesartan and amlodipine besylate (n=3)

Stress conditions	Duration	%Drug recovered (mean±SD)	% Drug decomposed (mean±SD)	Retention time (min) (mean±SD)	Theoretical plates (mean±SD)	Tailing factor (mean±SD)
Irbesartan						
Control Sample	----	99.96±1.21	----	6.231±1.22	3440±1.89	1.43±0.56
Acid Degradation	6 h	89.30±1.09	10.66±1.09	6.311±1.65	3598±1.73	1.52±0.89
Alkaline degradation	6 h	81.5±0.98	18.46±0.98	6.396±1.89	3462±1.56	1.39±0.45
Oxidative Degradation	2 h	69.54±1.65	30.42±1.65	6.210±0.36	3786±0.99	1.32±0.29
Thermal Degradation	2 d	94.9±1.53	5.06±1.53	6.222±0.87	3580±1.09	1.31±0.84
Photolytic Degradation	10 d	84.3±0.78	15.66±0.78	6.191±0.89	3582±1.54	1.56±0.99
Amlodipine Besylate						
Control Sample	----	100.06±1.56	----	5.789±0.66	5225±0.92	1.24±0.74
Acid Degradation	6 h	96.62±1.36	3.44±1.36	5.781±1.23	5853±0.39	1.19±0.52
Alkaline Degradation	6 h	69.62±1.57	30.44±1.57	5.852±0.78	5674±0.66	1.26±0.33
Oxidative Degradation	2 h	69.7±1.62	30.36±1.62	5.712±0.44	5281±0.98	1.21±0.92
Thermal Degradation	2 d	99.63±1.78	0.43±1.78	5.712±0.60	5736±0.79	1.21±0.78
Photolytic Degradation	10 d	71.34±1.41	28.72±1.41	5.743±0.61	5452±1.67	1.32±0.68

n is number of determinations, SD is standard deviation

DISCUSSION

The present RP-HPLC method is a simple, precise, specific, accurate, linear and robust for analyzing irbesartan and amlodipine besylate in the sample mixture. The results obtained from the above set of observations prove that the method is useful in qualitative and quantitative analysis of irbesartan and amlodipine besylate in dosage forms. There are some RP-HPLC methods reported for the estimation of irbesartan and amlodipine besylate in dosage forms and biological fluids but the methods have the drawbacks as not of stability indicating [46, 47], having long retention time and low linearity range [48]. In the present method, a mixture of sodium acetate buffer p^H 4.0 and acetonitrile in the ratio of 30:70 v/v as mobile phase found to be most suitable of analysis of irbesartan and amlodipine besylate, since the chromatographic peaks obtained were having good system suitability parameters. The method was validated according to ICH guidelines and results were in compliance of ICH guidelines. The linearity of the method had a good correlation with concentration and peak area. The correlation coefficient of irbesartan and amlodipine besylate was found to be 0.999, which indicates good linear relationship over concentration range 10-70 µg/ml and 8-32 µg/ml for irbesartan and amlodipine besylate respectively. The % RSD values in intra-day and inter-day precision study were found to be less than 2 for irbesartan and amlodipine besylate, which indicate the method was precise. The amount of drug recovery was 99.96 % for amlodipine besylate and 100.6 % for irbesartan. Hence, the present developed method was said to be suitable for the analysis of drugs in their pharmaceutical dosage form. The developed method is stability indicating in nature, use of economical mobile phase and short chromatographic time.

CONCLUSION

The proposed method for the simultaneous estimation of amlodipine besylate and irbesartan validated as per the international conference on organization (ICH) guidelines and it is simple, specific and reliable. The data generated from the forced degradation studies enabled the evaluation of amlodipine besylate and irbesartan stability under a variety of ICH recommended conditions. These data are valuable for the safety and potency assessment of a drug product. Furthermore, this simple and rapid RP-HPLC method can also be used successfully for the determination of amlodipine besylate and irbesartan in pharmaceutical formulations without any interference from the excipient.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally.

CONFLICT OF INTERESTS

Declared none

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