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SIMULTANEOUS ESTIMATION OF AMLODIPINE AND IRBESARTAN FROM THEIR MARKETED TABLET FORMULATION BY SIMPLE REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

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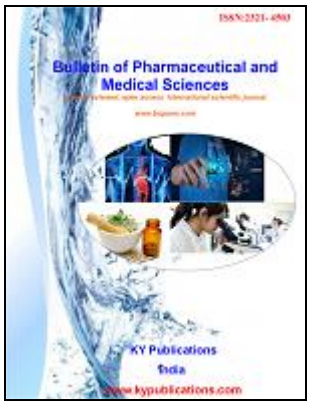

ABSTRACT
A simple, accurate, precise and rapid reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the simultaneous estimation of amlodipine and irbesartan in pure and tablet formulation. The separation of amlodipine and irbesartan was made on Agilent XDB C18 column (150×4.6mm,5µ) using the mobile phase containing phosphate buffer (pH-3.4) and acetonitrile in the ratio of 55:45v/v at a flow rate of 1.0 mL/min, at ambient column temperature (25°C), with detection at 245nm, and the injection volume was 10µl respectively. The method was statistically validated as per ICH guideline for analytical method validation. The validated method was used for simultaneous estimation of amlodipine and irbesartan from their marketed tablet formulation.

KEYWORDS: Amlodipine, Irbesartan, RP-HPLC, ICH guidelines.

A.INTRODUCTION:

Amlodipine [1,2] [(*RS*)-3-ethyl,5-methyl-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydro pyridine-3,5-dicarboxylate] (**Figure.1**) is a long-acting calcium channel blocker (dihydropyridine class) used as an anti-hypertensive and in the treatment of angina. Like other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart muscle.

Irbesartan[3-5](**Figure.2**),2-butyl-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]-1,3diazaspiro[4.4] non-1-en-4-one, is a non-peptidic angiotensin receptor blocker (ARB) mainly used for the treatment of hypertension. It exerts its antihypertensive by selectively blocking AT1 receptors that are abundantly present in vascular smooth muscle and inhibiting the strong vasoconstrictor action of angiotensin II.

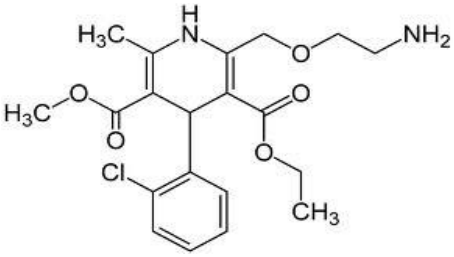

Fig.1.Structure of Amlodipine

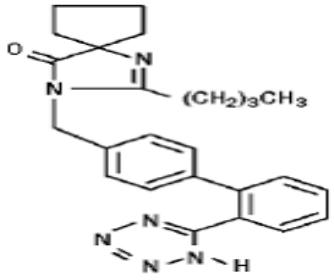

Fig.2.Structure of Irbesartan

These two drugs are available in the market for the treatment of hypertension either in alone or on combination. Very recently combinational drug [Amlodipine besylate and irbesartan] is introduced into the market that is particularly used treat patients with hypertension which is uncontrolled by usual doses of irbesartan or amlodipine besylate alone.

Literature survey revealed that few HPLC methods[6-15] have been reported for these drugs either in their individual forms or in combination with other drugs. Further, review of literature reported only two RP-HPLC methods have been reported for the simultaneous estimation of amlodipine and in combined dosage forms. In the view of their therapeutic importance in the present study, an HPLC method was optimized and validated for simultaneous estimation and validation of in amlodipine and irbesartan tablet formulation in accordance with the ICH guidelines.

B.EXPERIMENTAL:

a. INSTRUMENTATION: Chromatography was performed with Water's 2695 HPLC system provided with Hamilton Syringe, auto sampler and 2996 Photodiode array detector. The data acquisition, analysis and reporting were performed by Empower 2 (waters) chromatography software. Dig sun pH meter was also used for adjusting the pH of buffer solution. All weighing was done on sarotorious balance (model AE-160). Ultra-sonic bath sonicator was used for degassing and mixing of the mobile phase

b. REAGENTS AND CHEMICALS: Pharmaceutically pure sample of Amlodipine and Irbesartan were obtained from Spectrum Pharma Research Solutions, Hyderabad as gift samples along with their analytical reports. Commercial formulations (tablets) in the brand name of AIMIX® HD combination tablet containing 100mg of irbesartan and 10mg of amlodipine) was procured from the local pharmacy store. All the reagents including potassium dihydrogen phosphate, ortho phosphoric acid, HPLC grade Water, Acetonitrile and Methanol were purchased from Merck and Rankem Ltd. New Delhi, India.

c. MOBILE PHASE PREPARATION: Prepare a filtered and degassed mixture of phosphate buffer(pH-3.4) and acetonitrile in the ratio of 700:300 v/v respectively.

BUFFER PREPARATION: Dissolve 2.72g of Potassium dihydrogen Phosphate and 0.1% Tetra ethyl amine in 1000mL of Milli-Q Water, adjust pH to 3.4 with dilute ortho phosphoric acid and Filter the solution through 0.45µm membrane filter.

d. PREPARATION OF DILUENT: Mobile phase is used as diluent in the present assay.

e. PREPARATION OF STANDARD STOCK SOLUTION: Accurately weigh about 10mg of amlodipine and 100mg of irbesartan drugs into clean and dry 100ml volumetric flasks individually and dissolve in 100ml of diluent to get a concentration of 100µg/ml of amlodipine and 1000µg/ml of irbesartan (stock solution).Aliquot of 0.25ml, 0.5ml, 1.0ml, 1.25ml and 1.5ml and 2.5ml of this stock solutions were pipetted out and transferred into a series of 10ml volumetric flask separately and volume was made up to the mark with diluent to get concentrations of 12.5µg/ml, 25µg/ml, 50µg/ml, 62.5µg/ml, 75µg/ml and 125µg/ml for amlodipine and 125µg/ml, 250µg/ml, 500µg/ml, 625µg/ml, 750µg/ml and 1250µg/ml for irbesartan respectively.

f. PREPARATION OF SAMPLE SOLUTION: Twenty tablets of AIMIX HD mend for oral usage [containing 10mg of amlodipine and 100mg of irbesartan] were powdered and weighed accurately to get equivalent to 100mg of amlodipine and 100mg of irbesartan. The weighed powder was transferred to 100mL of dry volumetric flask and 60mL of diluent was added and sonicated for 15 minutes to dissolve the contents. The solution was made up to mark with diluent and finally filtered through 0.45 µm. Aliquots of this solution was transferred to a series of 10mL volumetric flask and diluted up to the mark with diluent used for HPLC, to obtain working sample solution of amlodipine and irbesartan that obey in linearity range.

C.RESULTS AND DISCUSSION:

i. METHOD DEVELOPMENT: Firstly spectroscopic analysis was carried out for the above two drug solutions in individual drug solutions of 125µg/ml prepared in different solvent mixtures of HPLC grade organic and inorganic solvents at different ratio. These drug solutions were then scanned in the UV region of 200-400 nm and the spectrums were recorded to get λmax. These drugs showed UV absorbance (λmax) at 242nm (For Amlodipine) and 255nm (For Irbesartan), respectively. The isoabsortive point of amlodipine and irbesartan was

observed at a λmax of 250nm and this wavelength was used for the present chromatographic detection of amlodipine and irbesartan using a photo diode array detector.

Further, a number of trials were made by changing the columns and mobile phase by varying its composition as well as by changing the solvents. All these trials have resulted either in low resolution or asymmetric peaks or peaks with more tailing factors or longer time of elution. However, finally the Aligent XDB C-18 column (150×4.6mm, 5µ) with a flow rate of 1.0mL/min of mobile phase and column temperature at 25°C with mobile phase of phosphate buffer(pH-3.4) and acetonitrile in the ratio of 55:45v/v had resulted in excellent elution of the two drugs with low retention and run times. The total run time of the present analysis was 6 minutes and the retention times of amlodipine and irbesartan were 3.849 and 2.72minutes respectively. The chromatograms obtained for the above said drugs during the method trials were represented in the **Figure.3**.

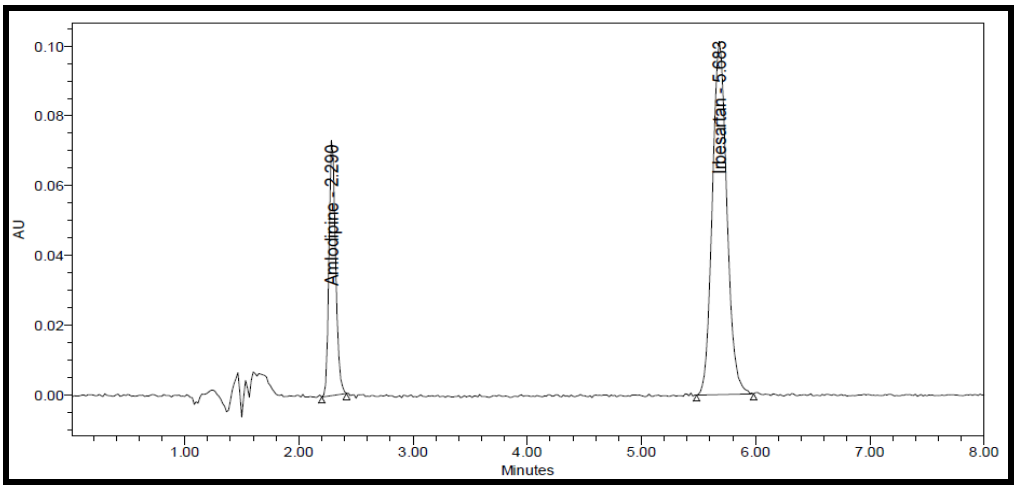

Fig.3. Standard chromatogram of Amlodipine and Irbesartan

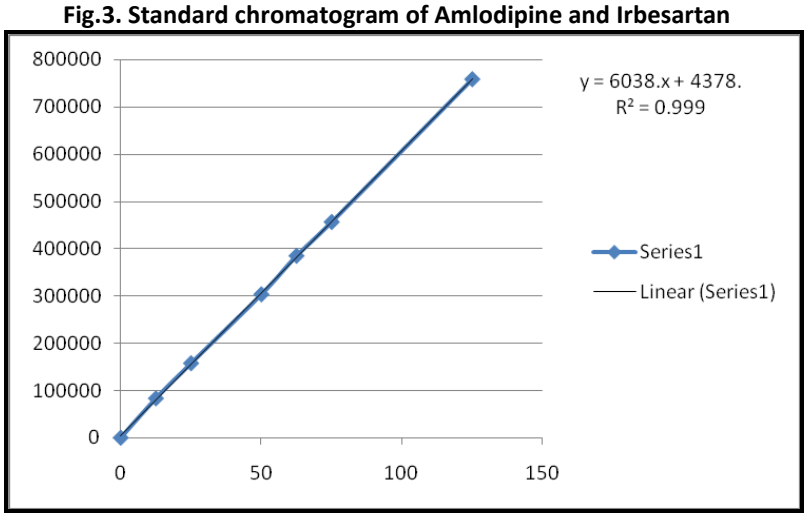

Fig.4.a Calibration curve of Amlodipine

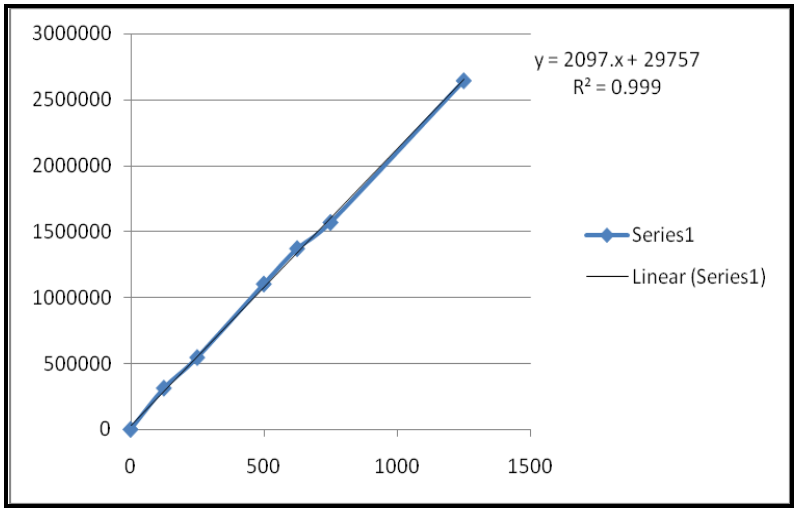

Fig.4.b Calibration curve of Irbesartan

CHROMATOGRAPHIC CONDITIONS: The optimized chromatographic conditions for the separation of amlodipine and irbesartan was made on Agilent XDB C_{18} column (150×4.6mm,5µ) using the mobile phase containing phosphate buffer (pH-3.4) and acetonitrile in the ratio of 55:45v/v at a flow rate of 1.0 mL/min, at ambient column temperature (25°C), with detection at 245nm, and the injection volume was 10µl respectively. Using the above optimized chromatographic conditions, validation studies was carried out further for the developed RP-HPLC method for amlodipine and irbesartan in combined dosage forms as per ICH guidelines.

iii. VALIDATION OF THE PROPOSED METHOD: The proposed method was validated as per ICH guidelines. The parameters studied for validation were system suitability, specificity, linearity, precision, accuracy, robustness, limit of detection, limit of quantification, and solution stability

a. SYSTEM SUITABILITY: The system suitability parameters established for the developed method include number of theoretical plates (efficiency), Resolution, Tailing factor. The HPLC system was equilibrated using the initial mobile phase composition, followed by six injections of the standard solution of 100% concentration containing 50µg/mL of amlodipine and 500µg/mL of irbesartan respectively. These six consecutive injections were used to evaluate the system suitability on each day of method validation. The results were given in the **Table.1**.

TABLE.1 SYSTEM SUITABILITY OF AMLODIPINE AND IRBESARTAN

PARAMETERS	AML	IRB
NO. OF THEORETICAL PLATES	7156	9358
TAILING FACTOR	1.26	1.08

TABLE.2.RESULTS OF LINEARITY STUDIES OF AMLODIPINE

PPM	SET-1	SET-2	SET-3	AVERAGE
12.5	83174	83238	83532	83315
25	159666	155675	157561	157634
50	309978	304571	295618	303389
62.5	389134	381113	384428	384891.7
75	464579	460095	444734	456469.3
125	763820	759229	752673	758574
SLOPE,(b)	6038			
INTERCEPT,(a)	4378.1			
CORRELATION, (r^2)	0.9999			
LOD	0.473704 µg/ml			
LOQ	1.4354µg/ml			

TABLE.3.RESULTS OF LINEARITY STUDIES OF IRBESARTAN

PPM	SET-1	SET-2	SET-3	AVERAGE
125	314994	312799	311278	313023.7
250	540703	547193	549583	545826.3
500	1104557	1103258	1100899	1102905
625	1371693	1374347	1370280	1372107
750	1567534	1566153	1576411	1570033
1250	2646219	2648247	2640559	2645008
SLOPE,b	2097			
INTERCEPT,a	29757			
CORRELATION, r^2	0.999			
LOD				
LOQ				

TABLE.4.RESULTS OF ACCURACY STUDIES OF AMLODIPINE AND IRBESARTAN

	SPIKED AMOUNT (PPM)		STANDARD DRUG SOLUTION (PPM)		% RECOVERED	
	AML	IRB	AML	IRB	AML	IRB
50%	25	250	50	500	101.8708	100.6913
	25	250	50	500	103.5581	102.6655
	25	250	50	500	102.0576	102.2375
100%	50	500	50	500	101.3375	101.4823
	50	500	50	500	99.3054	98.82928
	50	500	50	500	98.9162	101.672
150%	75	750	50	500	99.91874	100.3933
	75	750	50	500	98.98509	99.97914
	75	750	50	500	99.02595	99.98366
		MEAN*			100.5528	100.8815
		SD*			1.698345	1.23054
		%RSD*			1.689008	1.219787

*Average of three determinations

TABLE.5. RESULTS OF PRECISION STUDIES OF AMLODIPINE AND IRBESARTAN

VALIDATION PARAMETER	%MEAN		SD		%RSD	
	AML	IRB	AML	IRB	AML	IRB
REPEATABILITY	99.993	99.993	1.37439	1.560162	1.374481	1.560266
DAY-DAY	99.995	99.995	1.322131	1.564759	1.322197	1.564837

*Average of six determinations

TABLE.6. RESULTS OF ROBUSTENSS STUDIES OF AMLODIPINE AND IRBESARTAN

	CHANGED VALUE	RETENTION TIME		TAILING FACTOR		% ASSAY	
		AMD	IBS	AMD	IBS	AMD	IBS
COLUMN TEMPERATURE	25°C	2.47	5.82	1.23	1.07	101.36	101.70
	35 °C	2.25	5.41	1.17	1.06	100.68	100.56
FLOW RATE	0.9 mL/min	2.6	6.5	1.32	1.22	98.75	98.61
	1.1 mL/min	1.99	3.91	1.26	1.20	99.05	99.6
MOBILE PHASE	45:65 %v/v	2.23	5.38	1.28	1.20	102.26	101.74

COMPOSITION	65:45 %v/v	2.54	6.5	1.33	1.19	101.60	98.92
	Mean					100.61	100.52
	SD					1.42	1.22
	RSD					1.41	1.22

TABLE.7. STABILITY DATA OF AMLODIPINE AND IRBESARTAN

Drug	% ASSAY AT 0 Hr	% ASSAY AT 24 Hr
AML	100.75	100.95
IRB	100.26	101.47

TABLE.8.ASSAY OF AMLODIPINE AND IRBESARTAN IN FORMULATIONS

SAMPLE NO.	PEAK AREA		% ASSAY	
	AML	IRB	AML	IRB
1	296773	1018260	100.5976	100.8657
2	295209	1010124	100.0674	100.0598
3	301003	1018429	102.0314	100.8825
4	299778	1014135	101.6162	100.4571
5	292996	1002271	99.31729	99.28192
6	297580	1010186	100.8711	100.066
	AVG*		100.7502	100.2688
	SD*		0.994865	0.604502
	%RSD*		0.987457	0.602882

*Average of six determinations

b. SPECIFICITY: For this few studies were conducted to determine the specificity of the proposed method by injecting blank and placebo into the prescribed chromatographic system using the above defined chromatographic conditions their respective chromatograms were recorded. The chromatogram of the blank solution showed no peaks at the retention time of amlodipine and irbesartan peaks. Similarily chromatogram of the diluent solution showed no peaks at the retention time of amlodipine and irbesartan peaks revealing that the diluent used in the sample preparation did not interfere in estimation of amlodipine and irbesartan in combined dosage forms.

c. LINEARITY: Linearity was performed by preparing standard solutions of amlodipine and irbesartan at different concentration levels mentioned in experimental condition i.e., respectively. 10µl of each concentration was injected in triplicate into the HPLC system. The response was read at 250 nm and the corresponding chromatograms were recorded and tabulated (Table.2 & Table.3). From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually. The regressions of the plots were computed by least square regression method. The linearity response for both drugs amlodipine and irbesartain was between 125-1250µg/ml (Figure.4.a) and 12.5-250µg/ml (Figure.4.b) and the linearity were represented by the regression equation as shown below.

y(AML) = 6038.x + 4378(r^2 = 0.999) and y(IRB) = 2097.x + 29757 (r^2 = 0.999). From the data obtained in **Table.2&3** the proposed RP-HPLC method was found to be linear within the proposed range.

d. LOD AND LOQ: LOD and LOQ were determined by calibration curve method. For this amlodipine and irbesartan solutions were prepared in the concentration range of 12.5-125 and 125-1250µg/ml respectively and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations.

LOD= (3.3×Syx)/b, LOQ= (10.0×Syx)/b

Where Syx is residual variance due to regression; b is slope. LOD and LOQ for AML were 0.473704 and 1.435468µg/ml respectively and for IRB were 1.496712 and 4.535492µg/ml respectively.

e. ACCURACY: The accuracy of the present RP-HPLC method was determined by standard addition method performed at three concentration levels of 50%, 100% and 150%. This was carried out by addition of standard drug of amlodipine and irbesartan to the sample at 3 different concentration levels (50, 100 and 150%) in triplicate taking into consideration percentage purity of added bulk drug samples.. The percent recovery and %RSD at each level was calculated and results are presented in **Table.4.** Satisfactory recoveries ranging from 100.55% for amlodipine and 100.88% for irbesartan respectively were obtained by the proposed method indicating the good accuracy of the proposed method.

e. PRECISION:

i. REPEATABILITY: Six replicates of same concentrations of amlodipine and irbesartan were analyzed in same day for repeatability and the results were found to be within acceptable limits (RSD <2) as shown in **Table.5.**

ii. INTERMEDIATE PRECISION: Six replicates of same concentrations of amlodipine and irbesartan were analyzed on two different days and two analysts for day to day and analyst to analyst variation and the results were found to be within acceptable limits(RSD<2) as shown in table 2.5, revealing high precision of the proposed RP-HPLC method.

f. ROBUSTNESS: The robustness study was performed by slight modification in flow rate of the mobile phase, pH of the buffer and composition of the mobile phase. The samples of amlodipine and irbesartan were analyzed under these changed experimental conditions. The change was made in the ratio of mobile phase by ±10%, column temperature $\pm 5^0$c and the flow rate ±0.1mL. It was observed that there were no significant changes in the chromatographic elution and in peaks of amlodipine and irbesartan, when the above modifications were made in the experimental conditions, revealing the robustness of the developed method (**Table.6**).

g. STABILITY OF SAMPLE SOLUTION: The same sample solution of amlodipine and irbesartan was injected after 24hr did not show any appreciable change. Results are shown in **Table.7**.

ANALYSIS OF MARKETED FORMULATION: Analysis of marketed tablets was carried out using the above said optimized mobile phase and HPLC conditions. The % drug content of tablets obtained by the proposed method for amlodipine and irbesartan (Figure.3.10) was found to be 100.94 and 99.99 respectively. This showed that the estimation of dosage forms was accurate within the acceptance level of 95% to 100%. The results are given in **Table.8**.

D.CONCLUSIONS:

The developed RP-HPLC method provided selective quantification of amlodipine besylate and irbesartan without interference from diluent and placebo with added advantages of simple sample preparation and short analysis time [2.338 for amlodipine besylate and 5.813min for irbesartan] respectively. The results of linearity, precision, accuracy and robustness studies proved to be within the limits [as per International Conference on Harmonisation (ICH) Guidelines] making the proposed RP-HPLC method to be applicable for routine quality control analysis for the simultaneous estimation of amlodipine besylate and irbesartan using isocratic mode of elution.

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