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IMPROVEMENT AND LEGALIZATION OF A SIMPLE RP-HPLC METHOD FOR SIMULTANEOUS ASSESSMENT OF AMLODIPINE AND IRBESARTAN IN PHARMACEUTICAL DOSAGE FORMS

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Abstract - The aim of the study was to develop and validate high performance liquid chromatography (HPLC) assay for the simultaneous determination of amlodipine and irbesartan in multicomponent tablet dosage form. The experimental procedure involved reversed-phase-HPLC with buffer (pH-3.4): Methanol (55:45%v/v) as a mobile phase, at a stream pace of 1.0 ml/min. The stationary phase was the Aligent XDB C-18 column (150×4.6mm, 5µ). Amlodipine and irbesartan show most extreme retention at the wave length of 250nm was chosen as the discovery wave length. The retention times were seen as 2.3±0.5 min and 5.8±0.5 min for amlodipine and irbesartan individually. The method was validated with respect to specificity,precision, accuracy and linearity. Due to its simplicity and accuracy, the assay method is suitable for routine analysis of multi-component tablet formulation.

Keywords: HPLC, Amlodipine,Irbesartan, multi-component tablet formulation.

INTRODUCTION

Amlodipine (AML)[1-3] is artificially named as (RS)- 3-ethyl 5-methyl-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)- 6-methyl-1,4-dihydro pyridine-3,5-dicarboxylate (**Figure.1a**). Amlodipine (as besylate, mesylate or maleate) is a long-acting calcium channel blocker (dihydropyridine class) utilized as an enemy of hypertensive and in the treatment of angina. Irbesartan[4] (**Figure.1b**) is an angiotensin receptor blocker (ARB) utilized fundamentally for the treatment of hypertension. It rivals angiotensin II for official at the AT1 receptor subtype.

As per writing study there is no official strategies[5-7= for the synchronous estimation of amlodipine and irbesartan by RP-HPLC in joined tablet measurements structures. In this investigation, a new HPLC technique was enhanced and approved for synchronous estimation and approval of in amlodipine and irbesartan tablet plan as per the ICH rules.

EXPERIMENTAL

a.**INSTRUMENTATION:** Chromatography was performed with Water's 2695 HPLC framework furnished with Hamilton Syringe, auto sampler and 2996 Photodiode cluster finder. All HPLC frameworks were outfitted with a column compartment with temperature control and an on-line degasser. Information obtaining, analysis and revealing were performed by Empower 2 (waters) chromatography programming.

b.REAGENTS AND CHEMICALS: Pharmaceutically unadulterated sample of Amlodipine and Irbesartan were acquired from Spectrum Pharma Research Solutions, Hyderabad as blessing samples alongside their systematic reports. HPLC grade Water, Acetonitrile and Methanol were acquired from Ranchem and Commercial tablets of Amlodipine (10mg) and Irbesartan (100mg) AIMIX HD were secured from the nearby pharmacy store.

c.**CHROMATOGRAPHIC CONDITIONS:** The isocratic mobile phase comprised of Phosphate buffer: methanol (pH-3.4)in the proportion of 55:45%v/v at a stream pace of 1.0 ml/min. Agilent XDB C18 column(150×4.6mm,5µ) was utilized as stationery phase. The location wave length for both the medications is 250nm.

d. READINESS OF STANDARD STOCK SOLUTION: Accurately weigh about 10mg of Amlodipine and 100mg of Irbesartan drugs into spotless and dry 100ml volumetric flasks separately and break up in 100ml of diluent to get a convergence of 100µg/ml of Amlodipine and 1000µg/ml of Irbesartan (stock arrangement).

e. PLANNING OF WORKING STANDARD SOLUTIONS: Aliquot of 0.25ml, 0.5ml, 1.0ml, 1.25ml and 1.5ml and 2.5ml of stock arrangement were pipetted out and moved into a progression of 10ml volumetric flask independently and volume was made sufficient with diluent to get centralizations of 12.5 µg/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 separately.

f. PLANNING OF SAMPLE SOLUTION: Twenty tablets of AIMIX HD containing Amlodipine and Irbesartan (10mg and 100 mg separately) were gauged and powdered. The powder proportional to five tablets was moved into 100 ml volumetric flask. Break down the powder in 80ml of diluent, sonicated for 25 min. Afterward, the volume in the flask was made up to 100ml with diluent and separated. From this filtrate 1.0ml was pipetted out into a 10ml volumetric flask and make up the volume with diluent.

RESULTS AND DISCUSSION

i. STRATEGY DEVELOPMENT: Initially switch phase fluid chromatography partition was attempted to create utilizing different proportions of Methanol: water, Acetonitrile: water as mobile phase, in which both the medications didn't react appropriately, and the goals was additionally poor. The natural substance of the mobile phase was likewise examined to improve the partition of the two medications. To improve the following component, the pH of the mobile phase turns into a significant factor. At pH-3.4 the two medications eluted with better detachment.

ii. ENHANCEMENT OF THE DEVELOPED METHOD: Thereafter, buffer (pH-3.4): Methanol (55:45%v/v) was chosen as a mobile phase, at a stream pace of 1.0 ml/min. The stationary phase was the Aligent XDB C-18 column (150×4.6mm, 5µ). Amlodipine and irbesartan show most extreme retention at the wave length of 250nm was chosen as the discovery wave length. The maintenance times were seen as 2.3±0.5 min and 5.8±0.5 min for amlodipine and irbesartan individually. The chromatogram got was appeared in **figure.2**.

iii. **APPROVAL OF THE PROPOSED METHOD:** The proposed strategy was approved according to ICH rules[8]. The parameters read for approval were framework appropriateness, particularity, linearity, precision, exactness, strength, the point of confinement of discovery, farthest point of evaluation, and arrangement steadiness

a. FRAMEWORK SUITABILITY: System appropriateness parameters like the quantity of hypothetical plates, HETP and peak following were resolved. The qualities for the parameters were appeared in the **Table.1**.

b. EXPLICITNESS: An examination directed to build up particularity of the proposed strategy included infusing clear and fake treatment utilizing the chromatographic conditions characterized for the proposed technique. It was discovered that there is no obstruction due to excipients in the tablet detailing and furthermore found a decent correlation between the maintenance times of standard and sample.

c. LINEARITY: Linearity was performed by planning blended standard arrangements of amlodipine and irbesartan at various focus levels incorporating working fixation referenced in trial condition i.e., separately. 10µL of every focus was infused in triplicate into the HPLC framework. The reaction was perused at 250nm and the comparing chromatograms were recorded and organized (**Table.2a** and **Table.2b**). From these chromatograms, the mean peak areas were determined and linearity plots of

fixation over the mean peak areas were built exclusively. The regressions of the plots were registered by a least square regression technique. The linearity reaction for the two medications amlodipine and irbesartan was between 12.5-125µg/ml and 125-1250µg/ml (**Figure.3a**) and 12.5-250µg/ml (**Figure.3**) and the linearity were spoken to by the regression condition as demonstrated as follows. **y(AML) = 6038.x + 4378(r^2 = 0.999) y(IRB) = 2097.x + 29757 (r^2 = 0.999)**.

d. LOD AND LOQ: LOD and LOQ were determined by calibration curve method. 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 amlodipine were 0.4737 and 1.435µg/ml respectively and for amlodipine and irbesartan were 1.496 and 4.535µg/ml respectively.

e. PRECISION

i. REPEATABILITY: Six recreates of same fixations were investigated in same day for repeatability and results were seen as inside worthy points of confinement (RSD <2.0) as appeared in **Table.3**.

ii. TRANSITIONAL PRECISION: Six recreates of same focuses were dissected on two distinct days and two experts for everyday and examiner to investigator variety and results were seen as inside worthy limits (%RSD<2.0) as appeared in **Table.3**.

e. ACCURACY: The exactness of the strategy was controlled by the standard expansion technique. A known measure of standard medication was added to the fixed measure of pre-broke down tablet arrangement. Percent recuperation was determined by contrasting the area when the expansion of the standard medication. The standard expansion technique was performed at three focus levels of half, 100%, and 150%. The arrangements were broke down in triplicate at each level according to the proposed technique. The percent recuperation and %RSD at each level was determined and results are introduced in **Table.4.** Satisfactory recuperations extending from 98.91-103.55% for amlodipine and 98.82-103.6% for irbesartan separately were acquired by the proposed strategy. This shows the proposed strategy was precise.

f. ROBUSTNESS: The strength study was performed by slight alteration in stream pace of the mobile phase, pH of the buffer and arrangement of the mobile phase. The samples of amlodipine and irbesartan were broke down under these changed exploratory conditions. The changes were made in the proportion of mobile phase by ±10%, column temperature ±30ºC and the stream rate ±0.1mL/min. There were no critical changes in the chromatography design, when the above adjustments were made in the exploratory conditions, demonstrating that the technique is hearty (**Table.4)**.

g. STABILITY OF SAMPLE SOLUTION: The sample arrangement infused after 24hr didn't show any apparent change. The outcomes are appeared in **Table.6**.

h. TABLET ANALYSIS: Twenty tablets were gauged and determined the normal load of every tablet. At that point the tablets were powdered and weight equal to 5 tablets were moved into a 100mL volumetric flask, were moved into a 100mL volumetric flask, 80mL of diluent included and sonicated for 25min, further the volume made up with diluent and separated. From the sifted arrangement, 1ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluent. **Table.7** shows results got by the technique for the measure of amlodipine and irbesartan present in the tablets. The low estimations of %RSD show that the strategy is exact and precise.

CONCLUSIONS

A basic, quick, delicate and practical rp-hplc strategy has been created for the estimation of amlodipine and irbesartan and unadulterated and furthermore in consolidated dose structures. The believability of the proposed strategy has been built up by approval according to the ICH rules. The aftereffects of approval

were in great concurrence with satisfactory cutoff points. In this manner, the strategy has demonstrated to be exact, exact, linear, explicit and vigorous. subsequently it very well may be inferred that the proposed technique was a decent approach for getting dependable outcomes and saw as reasonable for the standard quality control analysis of amlodipine and irbesartan in unadulterated and furthermore in joined measurement structures.

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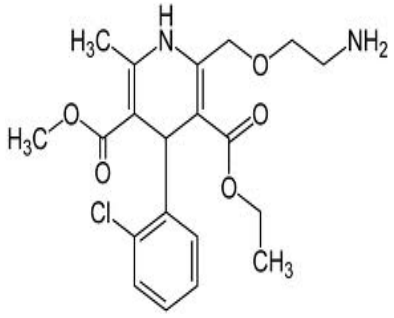

Fig.1a. Structure of Amlodipine

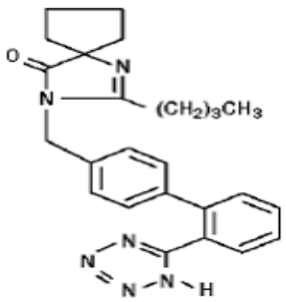

Fig.1b. Structure of Irbesartan

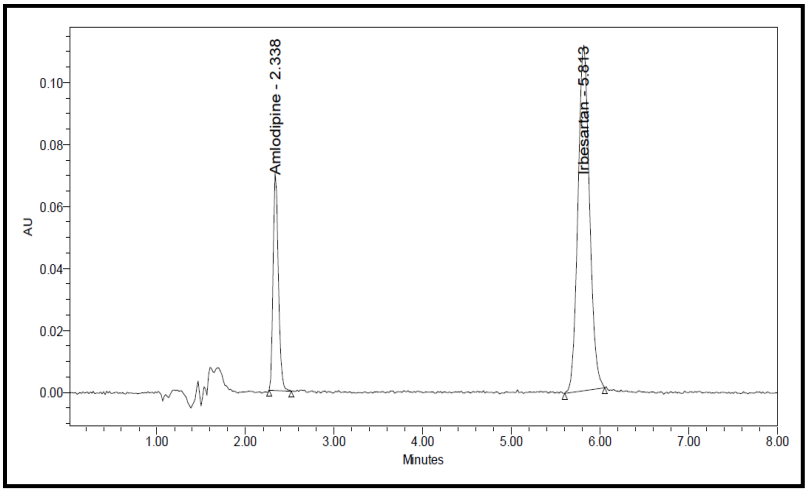

Fig.2 Typical Chromatogram of Amlodipine and Irbesartan

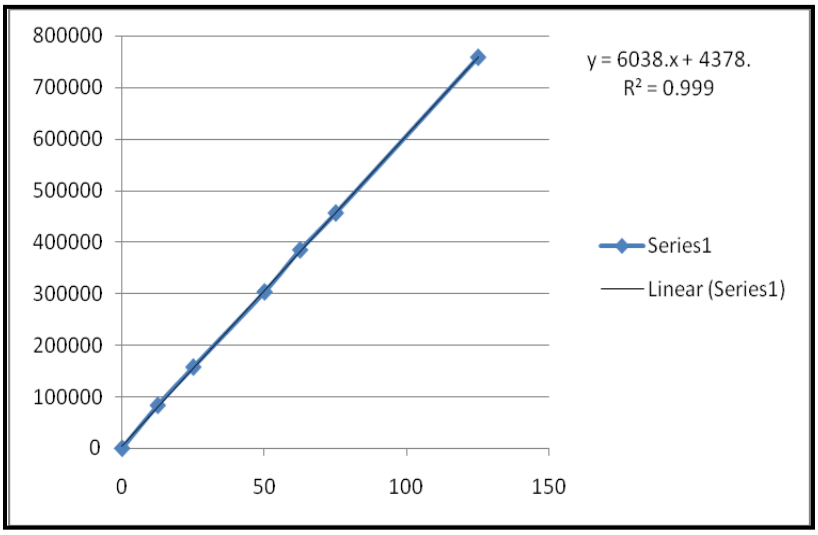

Fig.3a. Calibration curve of Amlodipine

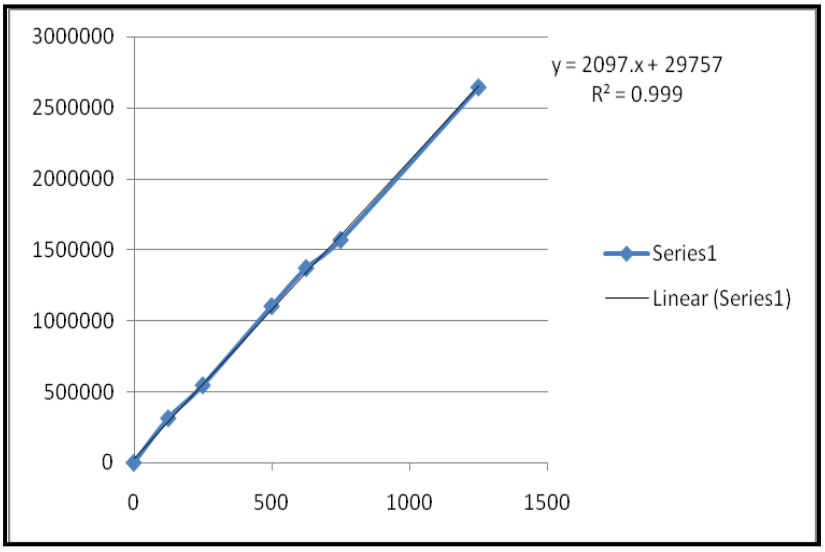

Fig.3b. Calibration curve of Irbesartan

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.a. RESULTS OF LINEARITY 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
Correlation, r^2				0.999
LOD(µg/mL)				0.473
LOQ(µg/mL)				1.435

TABLE.2.b. RESULTS OF LINEARITY 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(µg/mL)				1.497
LOQ(µg/mL)				4.535

TABLE.3.RESULTS OF PRECISION OF AML AND IRB

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

TABLE.4.RESULTS OF ACCURACY OF AML AND IRB

	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

TABLE.5. ROBUSTNESS DATA OF AMLODIPINE AND IRBESARTAN

	Changed value	Retention time		Tailing factor		% assay	
		AMD	IBS	AMD	IBS	AMD	IBS
Column Temperature	**25ºC**	2.47	5.82	1.23	1.07	101.36	101.70
	35 ºC	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 Composition	**65:35 %v/v**	2.23	5.38	1.28	1.20	102.26	101.74
	35:65 %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.6.STABILITY DATA OF AML AND IRB

Drug	% Assay at 0 hr	% Assay at 24 hr
AML	100.75	100.95
IRB	100.26	101.47

TABLE.7. RESULTS FOR HPLC ANALYSIS OF TABLETS

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