JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

"RP-HPLC METHOD DEVELOPMENT AND SIMULTANIUS ESTIMATION OF AMLODIPINE BESYLATE AND IRBESARTAN"

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ABSTRACT

To perform an assessment of a commercial tablet formulation, a reverse phase high performance liquid chromatography (RP-HPLC) method was created, verified, and estimated simultaneously to measure the stability of irbesartan and amlodipine in combination dose form at a single wavelength (245nm) (AIMIX Tablet). The orthophoshoric acid buffer was used to develop the isocratic technique on an HPLC hypersil BDS (Shimadzu-LC 20AT) C18 column with 4.6 mm i.d. and 5 m particle size: 1.0 mL/min of chloroform, toluene, methanol, and acetic acid, supplied at ambient temperature (25 oC) and injection volume (20 l), respectively. Amlodipine besylate and irbesrtran both had average peaks of 1477.436 and 3622.950, respectively. The analysis's findings were statistically confirmed in accordance with ICH recommendations. Based on the findings of the linearity range and RSD for intraday precision, the suggested RP-HPLC technique was determined to be sensitive, accurate, precise, simple, and quick. All of the results were satisfactory, demonstrating the method's viability for use in daily quality control and drug testing.

Keywords: Amloldipine Besylate, Irbesartan, Combined Dosage Form

INTRODUCTION

Chemically, amlodipine besylate is known as 2-[(2- aminoethoxy)-methyl]. -4-(2-chlorophenyl) 1,4-dihydro-6-methyl-3,5- pyridine-dicarboxylic acid-3 ethyl-5 methyl ester and irbesartan, chemically defined as 2-butyl-3-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1,3-diazaspiro[4.4]non-1-en-4-one. Irbesartan and amlodipine besylate have molecular weights of 408.879 and 428.53 respectively. Irbesartan and its active metabolite have an 8500-fold higher affinity for the AT1 receptor than the AT2 receptor. Irbesartan lowers blood pressure by preventing the binding of angiotensin II, which relaxes vascular smooth muscle and prevents the release of aldosterone. Amlodipine is a calcium channel antagonist with a long half-life that targets calcium ion influx across membranes and inhibits it. The different pharmaceuticals and drug-related degradants that can occur during storage or production should be able to be separated, detected, and quantified by HPLC methods. These

methods should also be able to detect and quantify any drugs and drug-related impurities that may be added during synthesis. The process of determining a method's performance qualities and limitations, as well as the factors that may affect them and to what degree, is known as validation. The separation method known as high-performance liquid chromatography (HPLC) can be Depending on the kind of stationary phase utilised, HPLC is based on mechanisms of adsorption, partition, and ion exchange.

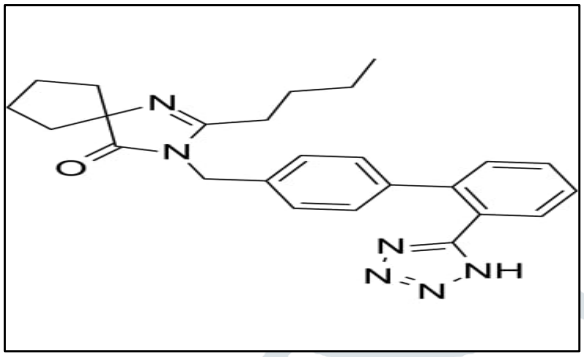

Fig. 1: Chemical Structure of Irbesartan

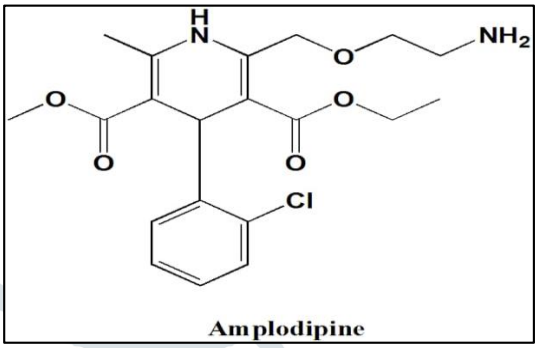

Fig. 2: Chemical Structure of Amlodipine Besylate

MATERIALS AND METHOD

CHEMICALS AND REAGENT

- Standard drug samples of amlodipine besylate and irebesartan. (Formulation: AIMIX Tablet)
- Toluene – AR Grade (Merck India Limited)
- Ethyl acetate- AR Grade (Allied Chemical Corporation, Vadodara, Gujarat, India.)
- Methanol (Allied Chemical Corporation, Vadodara, Gujarat, India)
- Chloroform (Allied Chemical Corporation, Vadodara, Gujarat, India)
- Glacial Acetic Acid (Allied Chemical Corporation, Vadodara, Gujarat, India)
- TLC Aluminum sheet percolated with silica gel 60 F254 (20×20cm^2,Merck India Limited)

APPARATUS AND EQUIPMENT

- HPLC (Shimadzu-LC 20AT)
- C18 column (250 mm × 4.6 mm i.d., particle size 5 µm)
- Camag Linomat V (Semiautomatic Spotting device)
- Camag Twin Tough Chamber (10 × 10 cm2)
- Camag TLC Scanner-3
- Camag win CATS v.1.3.4 Software
- Hamilton Syringe (100 µl)
- Digital weighing balance– Denver SI234, Germany
- Volumetric flask – 10 ,25 and 100 ml
- Pipettes – 1, 2, 5 and 10 ml

Chromatographic System

- **Stationary phase:** Pre-coated Silica gel G60 F254 aluminum Sheets 10×10 cm2, layer thickness 0.2 mm
- **Mobile phase:** Ethyl Acetate: Chloroform: Toluene: Methanol: Acetic acid (5:5:3:0.4 v/v/v/v.)
- **Temperature:** Ambient

PREPERATION OF STANDARD STOCK SOLUTION

Preparation of AML standard stock solution

Accurately weighed 10 mg of AML was transferred into 10 ml volumetric flask and dissolved in methanol and diluted up to the mark with methanol to get a stock solution having concentration of 1 mg/ml (1000 µg/ml).

Preparation of AML working standard solution.

1.0 ml of AML standard stock solution was diluted to 10 ml with methanol to get AML working standard solution having concentration of 100 µg/ml.

Preparation of Irbesartan standard stock solution

Accurately weighed 10 mg of irbesartan was transferred into 10 ml volumetric flask and dissolved in methanol and diluted up to the mark with methanol to get a stock solution having concentration of 1 mg/ml (1000 µg/ml).

Preparation of Irbesartan working standard solution.

1.0 ml of irbesartan standard stock solution was diluted to 10 ml with methanol to get AML working standard solution having concentration of 100 µg/ml.

Preparation of solution for calibration curve of Irbesartan and Amlodipine.

10ml of Amlodipine and10ml of irbesartan standard solution was transferred into 100 ml volumetric flask and diluted up to the mark with methanol. From that 3, 4, 6, 8, 10 and 12µl was spotted on to the plate to get the concentration range of 300-1200ng/spot for all three drugs.

Preparation of sample solution of marketed formulation (Irbesartan and Amlodipine combination tablet) [71]

Content of twenty tablets were weighed accurately. A powder quantity equivalent to 10 mg Amlodipine and 150 mg irbesartan was accurately weighed and transferred to volumetric flask of 10 ml capacity. 7 ml of Methanol was transferred to this volumetric flask and sonicated for 20 min. The flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through whattman filter paper (0.45µ). Filtrate 1ml was transferred to 10ml volumetric flask and diluted up to the mark with methanol to get a solution containing 100 µg/ml of amlodipine, 150 µg/ml of irbesartan. The resultant solution (3µl) was spotted on the plate so that concentration of amlodipine was 300ng/spot, irbesartan was 1200ng/spot.

WAVELENGTH FOR DETECTION

245nm wavelength was selected for estimation of this combination.

METHOD VALIDATION

The analytical method was validated as per the Q2 of the International Conference on Harmonization (ICH) (R1) guidelines for system suitability, linearity, accuracy, precision, limit of detection, limit of quantitation and robustness.

RESULT AND DISCUSSION

Optimization of Chromatographic Conditions:

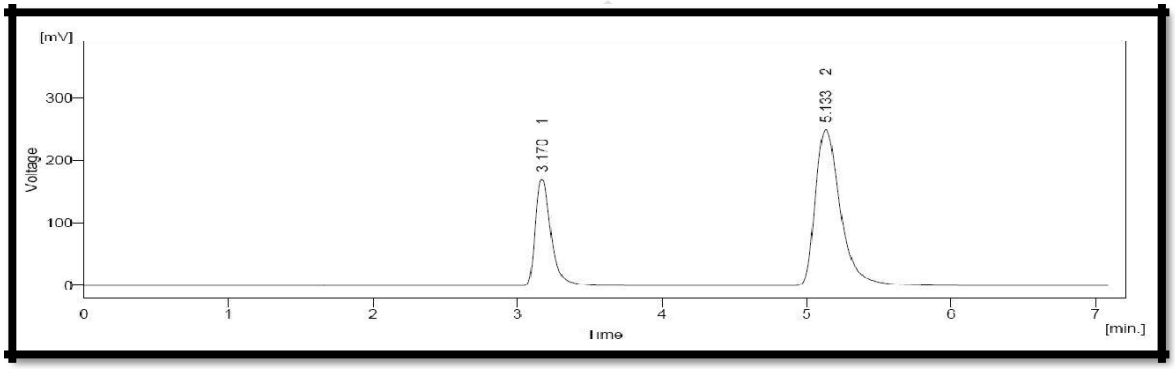

Fig.3: Optimized chromatogram of Amlodipine besylate and Irbesartan.

Table 1: Optimized chromatographic conditions:

Parameters	Condition
Stationary phase	Hypersil BDS C18 column (250mm X 4.6 mm i.d., 5 µm particle size)
Mobile phase	Water(pH-3.5): ACN (60 : 40)
Pump mode	Isocratic
Flow rate (ml/min)	1.0
Run time (min)	10.0
Volume of injection (µl)	20
Detection wavelength (nm)	245

METHOD VALIDATION

Linearity:

Calibration curve for the Amlodipine besylate (5-15 µg/mL):

Conc (µg/mL) (n=6)	Area (mean ± S.D.)
5	741.042
7.5	1107.724
10	1478.971
12.5	1792.845
15	2216.245

Table 2: Calibration curve for Amlodipine besylate

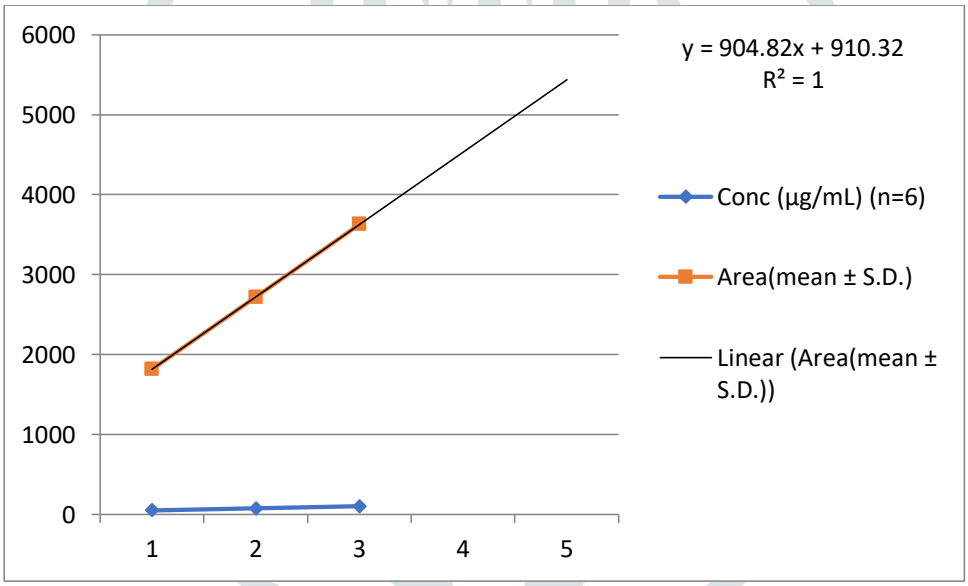

Fig.4: Graph of calibration curve of Amlodipine besylate

Linearity range for Amlodipine bisulfate was found to be 5-15 µg/ml in Mobile Phase. Regression Equation for Amlodipine besylate 245 nm: Y=145.4x-+13.15 r² value: 0.998.

Calibration curve for the Irbesartan (50-150 µg/mL):

Conc (µg/mL) (n=6)	**Area(mean ± S.D.)**
50	1816.935
75	2716.369
100	3626.576

Table 3**:** Calibration curve for Irbesartan

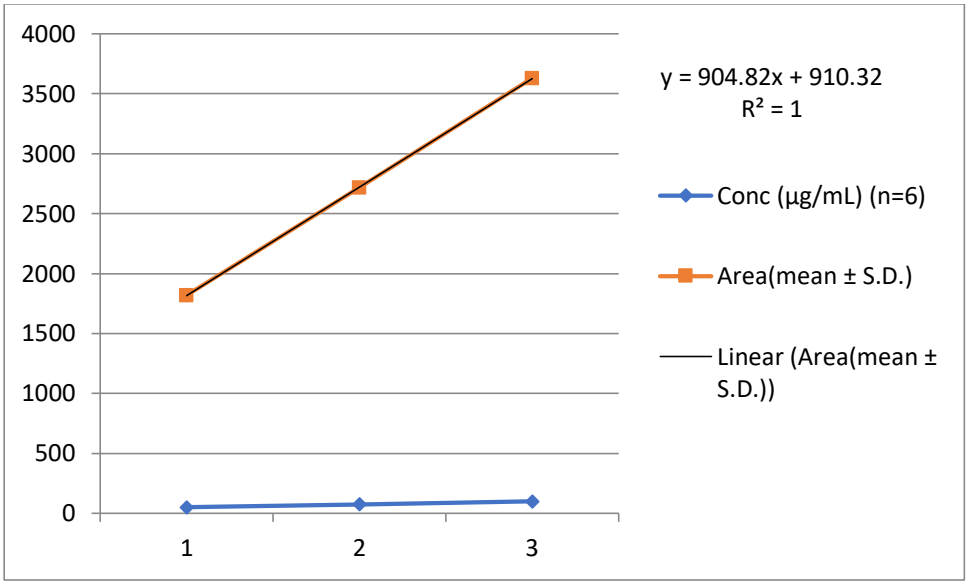

Fig.5: Graph of Calibration curve for Irbesartan

Linearity range for Irbesartan was found to be 50-150 µg/ml in mobile phase. RegressionEquation for Irbesartan at 245 nm.Y= 35.68x + 31.3, r ²value: 0.998.

Accuracy (% Recovery study):

%Spiking	Amount of test taken(µg/ml)	Amount if standard added(µg/ml)	Total amount of conc.	Total conc found(µg/mL)	Calculated spiking conc (µg/ml)	Mean % Recovery ±SD
80 (n=3)	5	4	9	8.93	3.93	99.596 ± 0.80
100 (n=3)	5	5	10	10.02	5.02	99.545 ± 0.628
120 (n=3)	5	6	11	11.13	6.13	100.27 ±91.028

Table 4: %Recovery data for Amlodipine besylate

%Spiking	Amount of test taken (µg/mL)	Amount of std added (µg/mL)	Total amount of Conc.	Total conc. Found (µg/mL)	Calculated spiking Conc. (µg/mL)	Mean % Recovery ± SD
80 (n=3)	50	40	90	90.01	40.01	100.10 ± 0.285
100 (n=3)	50	50	100	99.99	49.99	99.89 ± 1.384
120 (n=3)	50	60	110	110.36	60.36	101.59 ± 0.176

Table 5: %Recovery data for Irbesartan

Precision:

Repeatability:

Table 6: Repeatability data for Amlodipine besylate and irbesartan.

Drug	Target Conc. (µg/mL)	Peak Area of Sample	Mean	SD	%RSD
Amlodipine besylate	10	1495.254	1484.49	6.90	0.46
	10	1478.25			
	10	1485.235			
	10	1489.414			
	10	1481.254			
	10	1477.554			

Irbesartan	100	3674.541	3664.447	23.17	0.63
	100	3679.548			
	100	3620.127			
	100	3680.248			
	100	3675.245			
	100	3659.447			

Intraday precision:

Table 7: Intraday precision data for Amlodipine besylate and irbesartan.

Drug	**Target Conc.** (µg/mL)	**Peak Area of Sample**	**Mean**	**SD**	**%RSD**
Amlodipine besylate	5	746.236	740.562	5.24	0.70
	5	735.880			
	5	739.571			
	10	1495.258	1478.317	16.31	1.10
	10	1462.699			
	10	1476.995			
	15	2238.427	2214.749	22.32	1.00
	15	2194.092			
	15	2211.728			
Irbesartan	50	1829.709	1815.818	12.89	0.71
	50	1804.218			
	50	1813.528			
	100	3673.939	3629.107	43.65	1.20
	100	3586.731			
	100	3626.657			
	150	5487.738	5422.734	57.34	1.05
	150	5379.30			
	150	5401.165			

Interday precision:

Table 8: Interday precision data for Amlodipine besylate and Irbesartan

Drug	Target conc(µg/ml)	Peak area of sample.	Mean	SD	%RSD
Amlodipine besylate	5	734.409	743.296	8.9	1.19
	5	752.209			
	5	743.270			
	10	1498.769	1482.821	19.94	0.94
	10	1467.127			
	10	1487.566			
	15	2189.684	2220.693	27.67	1.24
	15	2242.867			
	15	2229.528			
Irbesartan	50	1800.564	1819.464	22.45	1.23
	50	1844.293			
	50	1813.535			
	100	3662.943	3637.539	35.02	0.96
	100	3597.588			
	100	3652.086			
	150	5368.188	5439.131	65.97	1.21
	150	5498.655			
	150	5450.551			

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Table 9: LOD and LOQ data for Amlodipine besylate and Irbesartan.

Parameters	Amlodipine besylate	Irbesartan
Mean Slope (n=6)	0.185	0.010
SD of Y intercept (n=6)	0.0023	0.0027
LOD(µg/mL)	0.41	0.856
LOQ(µg/mL)	1.24	2.59

Robustness Study:

Change in flow Rate:

Table 10: Robustness data for Amlodipine besylate and Irbesartan with change in flow rate.

Parameter	Amlodipine besylate(%RSD)	Irbesartan(%RSD)
Folw rate(+ 0.2)0.8ml/min	0.8646	0.8839
Flow rate(- 0.2)1.2ml.min	0.6506	0.6443
Mobile phase (62:38)	0.9626	1.067
Mobile phase (58:42)	0.8144	1.067
pH (- 0.2) 3.3	1.2457	1.3549
pH (+0.2) 3.7	0.9018	1.010

System suitability:

Table 11: System suitability Parameters

Name	Rt (min)	Area	Tf	Resolution	Theoretical Plate#
Amlodipine besylate	3.170	1263.60	1.6	7.788	4334
Irbesartan	5.133	2997.67	1.69		4343

Analysis of Pharmaceutical Preparations:

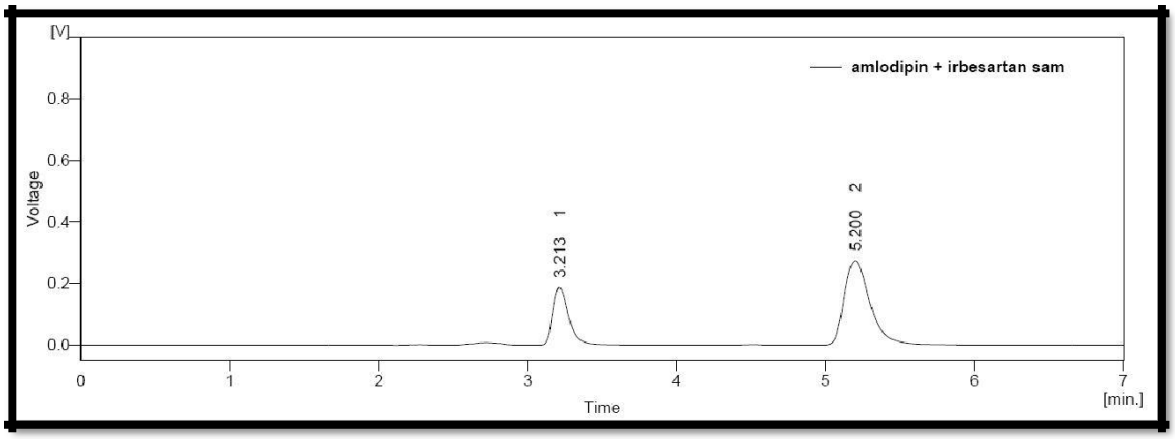

Fig. 6: %Assay of Amlodipine besylate (10µg/mL) and Irbesartan (100µg/mL) in their tablet dosage form

Table 12: Chromatograph of 10 µg/mL of Amlodipine besylate and 100µg/mL of Irbesartan prepared from tablet (AIMIX TAB)

AIMIX TAB	**Label claim mg/tablet**	**Conc. taken for assay (µg/mL)**	**Ave. Peak area of sample***	**Conc. Found form Tablet (µg/mL)***	**% Assay* ± SD**
AMLO	10 mg	10 (µg/mL)	1477.436	10.09	100.95
IRBE	100 mg	100 (µg/mL)	3622.950	100.66	100.66

CONCLUSION

The simultaneous determination of combination anti-hypersensitive amlodipine besylate and irbesartan using the suggested RP-HPLC method was found to be sensitive, accurate, precise, easy to use, and quick. The majority of the work should go into method development and optimisation when creating an HPLC method because doing so will enhance the performance of the finished method. It was discovered that specific chromatographic conditions may distinguish between irbesartan (Rt = 5.133) and amlodipine besylate (Rt = 3.170) with a resolution of 7.778. The methods were validated for linearity, accuracy, precision, limit of detection, limit of quantification, and sensitivity in accordance with ICH criteria. For routine examination of the raw ingredients in combinational dose formulations combining amlodipine besylate and irbesartan, the present RP-HPLC method can therefore be utilised.

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