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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD OF BISOPROLOL AND AMLODIPINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT The objective of the recent study was to develop a simple, accurate and precise RP-HPLC method with subsequently validate as per ICH guidelines for the determination of Amlodipine (AMLO) and Bisoprolol Fumarate (BISO) using mobile phase (mixture of a Methanol: Water (0.1%OPA) 65:35 v/v) as the solvent. The proposed method involves the measurement of Retention time at analytical wavelength 230 nm was selected. The Retention time of Amlodipine and Bisoprolol Fumarate was found to be 3.49 and 6.52 min respectively. The linearity of the proposed method was investigated in the range of 5-25 µg/ml for both the drugs Amlodipine and Bisoprolol Fumarate. The method was validated for its linearity, accuracy and precision. Both inter-day and intra-day variation was found to be showing less 2 % RSD.

KEYWORDS : RP-HPLC method, Amlodipine, Bisoprolol Fumarate, Validation.

INTRODUCTION

Pharmaceutical Analysis plays a vital role in quality assurance and quality control of bulk drugs and their formulations. Pharmaceutical analysis is a particular branch of analytical chemistry, which includes isolating, identifying and determining the relative amounts of compounds in a sample matter. It is concerned with chemical characterization of matter both quantitative and qualitative. In recent years many analytical techniques have been developed. Analytical method is a particular utilization of a procedure to solve a problem. Analytical instrumentation assumes an imperative part in the production and evaluation of new products and protection of Consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, medications, water and air.

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. There are two important reasons for validating assays in the pharmaceutical industry. The first, and by for the most important, is that assay validation is an integral part of the quality control system. The second is that current good manufacturing practice regulation requires assay validation.

Bisoprolol Fumarate **Fig. 1** is chemically (RS) -1- {4-[(2 – isopropoxyethoxy) methyl] phenoxy } -3- (isopropyl amino) propan -2-ol. It is β1 selective 2nd generation drug. B – blocker lacking intrinsic sympathomimetic activity; suitable for once daily administration in angina, hypertension and CHF. It is official in United State Pharmacopoeia. . It is freely soluble in ethanol and methanol. Molecular formula of Bisoprolol Fumarate is (C18H31NO4)2. C4H4O4 and molecular weight is 766.96 g/mol.

Amlodipine **Fig. 2** is chemically a 2-[(2-Aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid-3-ethyl 5-methyl ester and it belongs to the class of calcium channel blocker [1-5].

Literature review reveals that several methods such as HPLC, HPTLC, UV Spectrophotometry, UPLC etc [7-20]. Methods have been reported for the individual drugs as well as in combination with others drugs in formulation. But no method was reported for the simultaneous estimation of Cilnidipine and Bisoprolol Fumarate in tablet dosage form by HPLC method. Therefore main objectives of study were to develop simple, accurate and precise method for estimation of Cilnidipine and Bisoprolol Fumarate. Validation of the developed method done in accordance with ICH guidelines [6].

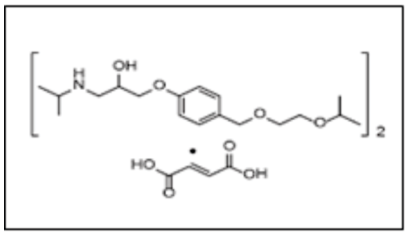

Fig. no.1- Structure of Bisoprolol Fumarate

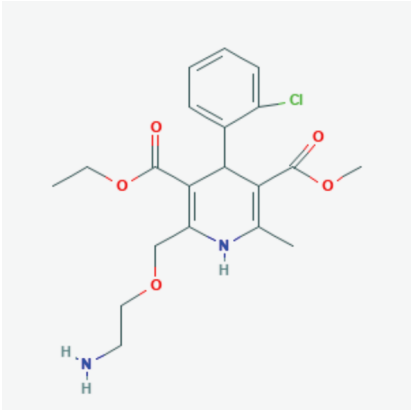

Fig. 2: Structure of Amlodipine

MATERIALS AND METHODS

Materials and Reagents

The analysis of the drug was carried out on Agilent (S. K.) Gradient System UV Detector. Equipped with reverse phase (Grace) C_{18} column (4.6mm x 250mm; 5µm), a SP940D pump, a 20µl injection loop and UV740D Absorbance detector and running Chemstation software. Amlodipine and Bisoprolol Fumarate were procured from R.S.I.T.C Jalgaon. Orthophopsphoric acid (OPA) (Avantor Performance

material India Ltd. Thane, Maharashtra) and methanol, acetonitrile, (HPLC grade Merck Specialties Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai.), water, 0.45 µm filter (Millipore, Bangalore). A combination of Amlodipine (2.5 mg) and Bisoprolol Fumarate (5 mg) in tablet formulation (CONCOR AM 5 MG) was procured from Meck limited from the local market.

Chromatographic Conditions

Column C_{18} (250 mm× 4.6 mm); particle size packing 5µm ; detection wavelength of 230 nm; flow rate 0.1 ml/min; temperature ambient; sample size 20 µl; mobile phase methanol: water (OPA 0.1%) (65:35); run time of 15 min.

Preparation of standard stock solution

10 mg of Amlodipine and 10 mg of Bisoprolol Fumarate were weighed accurately and transferred to a 10 ml volumetric flask dissolved in methanol and diluted to 10 ml with the mobile phase methanol + 0.1% OPA water (65 + 35% v/v) to give a stock solution of 1000 µg/ml Amlodipine and 1000 µg/ml Bisoprolol Fumarate**.** Sample were injected and peaks were recorded at 230 nm as the graph plotted as concentration of drug verses peak area is depicted in **Fig. 3**.

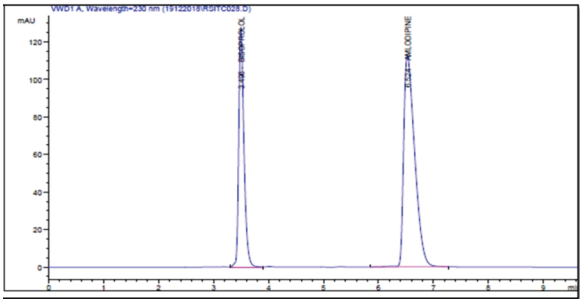

Fig. 3: Chromatogram of standard combination of AMLO and BISO

Assay Preparation For Commercial Formulation

For analysis of the tablet dosage form, Weigh 20 Amlodipine and Bisoprolol combination tablets and calculated the average weight, accurately weigh and transfer the sample equivalent to 5.6 mg Amlodipine and Bisoprolol into 10 ml volumetric flask. Add about 10ml MEOH of diluents and sonicate to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 0.45 µm filter. Further pipette 0.1ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents. (10 µg/ml). The simple chromatogram of test Amlodipine and Bisoprolol Shown **Fig. 4.** The amounts of Amlodipine and Bisoprolol per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet Assay for %Label claim for %RSD Calculated, Result was shown **Table 1.**

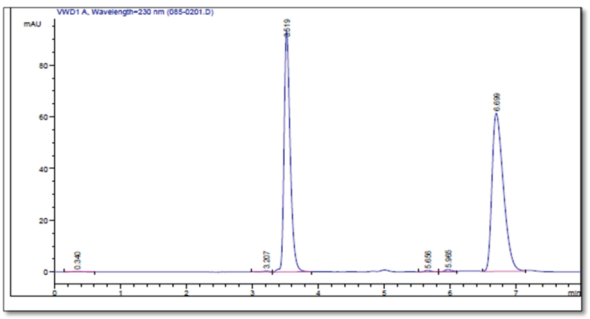

Fig. 4: Chromatogram for marketed formulation

Table 1: Analysis of marketed formulation

Assay	Drug	Amt. Found	% Label Claim	SD	%RSD
Rp-HPLC Method	AMLO	40.30	100.77	1.07	0.03
	BISO	80.71	100.89	0.72	0.14
	AMLO	40.35	100.88	0.03	0.11
	BISO	80.67	100.84	0.04	0.08

METHOD DEVELOPMENT AND VALIDATION

Serial dilutions were done to prepared various concentration stock (Standard solution and diluted to get required concentration for calibration plot and which was injected [21-30].

RESULTS

LINEARITY AND RANGE

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 5-25µg/ml for Amlodipine and 5-25µg/ml for Bisoprolol **Table 2 and 3** depict the calibration data of Amlodipine and Bisoprolol. The respective linear equation for Amlodipine was y = 29.518 x + 2.72. The correlation coefficient was 0.9998**.** The respective linear equation for Bisoprolol was equation y = 23.251 x + 5.675. Where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Amlodipine and Bisoprolol is depicted in **Figure 5 and 6.**

Table 2: Linearity data for Amlodipine

Method	Conc. µg/ml	Peak area (µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	5	144.89	144.16	144.525	0.52	0.36
	10	308.89	308.75	308.82	0.10	0.03
	15	444.37	443.88	444.125	0.35	0.08
	20	585.94	586.5	586.22	0.40	0.07
	25	743.87	743.68	743.775	0.13	0.02
	Equation		y = 29.518 x + 2.72			
	R^2		0.999			

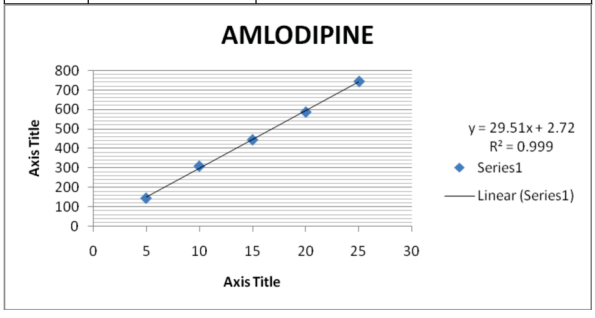

Fig. 5: Calibration curve of Amlodipine

Table 3: Linearity data for Bisoprolol

Method	Conc. µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	5	116.59	116.52	116.555	0.05	0.04
	10	246.99	246.34	246.665	0.46	0.19
	15	353.88	354.22	354.05	0.24	0.07
	20	467.37	467.85	467.61	0.34	0.07
	30	586.86	587.87	587.365	0.71	0.12
	Equation		y = 23.251 x + 5.675			
	R^2		0.9992			

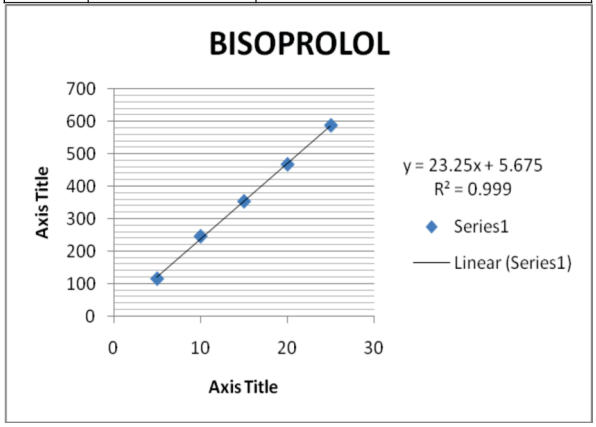

Fig. 6: Calibration curve of Bisoprolol

ACCURACY

Recovery studies were performed to validate the accuracy of developed method. To a pre-analysed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed**.** The % recovery was found to be within 98-101%. Statistical validation of recovery studies are shown in **Table 4.**

Table 4: Statistical Validation of Recovery Studies Amlodipine and Bisoprolol

Level of Recovery (%)	Drug	% RSD	S. D.*	Mean % Recovery
80%	**AMLO**	0.81	0.81	100.07
	BISO	0.92	0.92	99.86
100%	**AMLO**	0.14	0.14	99.90
	BISO	0.24	0.24	101.15
120%	**AMLO**	1.13	1.13	99.97
	BISO	1.13	1.13	99.97

***Denotes average of three determinations for RP-HPLC method**

SYSTEM SUITABILITY PARAMETERS

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of AMLO and BISO system suitability parameters were studied. The result shown **Table 5.**

Table 5: Repeatability studies on RP-HPLC for Amlodipine and Bisoprolol

Conc. of AMLO and BISO (mg/ml)	Peak area	Amount found (mg)	% Amount found
15	443.86	14.95	99.67
15	444.03		
Mean	**443.95**		
SD	**0.12**		
%RSD	**0.03**		
15	360.73	15.25	101.67
15	360.12		
Mean	**360.43**		
SD	**0.43**		
%RSD	**0.12**		

PRECISION

The method was established by analyzing various standards of AMLO and BISO. All the solution were analyzed thrice in order to record any intra-day & inter-day variation in the result. The result obtained for interday and intraday variation are shown in the **Table 6.**

Table 6: Result of Intraday and Inter day Precision studies for AMLO and BISO

METHOD	Drug	Conc. (µg/ml)	Intraday Precision		Interday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
RP-HPLC Method	AMLO	10	303.53 ±0.96	102.27	303.07± 0.96	101.70
		15	433.81± 0.75	97.80	437.26± 0.39	98.13
		20	587.59± 0.59	98.40	589.17± 0.69	99.40
	BISO	10	244.29± 0.96	102.30	243.57 ±0.96	102.63
		15	351.3± 0.50	99.100	351.14± 0.64	99.06
		20	471.08± 1.79	100.40	472.61 ±0.80	100.05

***Mean of each 3 reading for RP-HPLC method**

ROBUSTNESS

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate on retention time and tailing factor of drug peak was studied. The results indicate that less variability in retention time and tailing factor were observed **Table 7.**

Table 7: Result of Robustness Study of Amlodipine and Bisoprolol

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	% RSD	Amount of detected (mean ±SD)	% RSD
		For AMLO		**For BISO**	
Chromatogram of flow change 0.9 ml	25	651.41	0.20	518.02	0.40
Chromatogram of flow change 1.1 ml	25	866.68	0.11	682.36	0.20
Chromatogram of comp change wavelength change 229nm	25	705.7	0.26	491.3	0.68
Chromatogram of comp change wavelength change 231nm	25	760.61	0.36	728.69	0.46
Chromatogram of mobile phase change 64+36 ml	25	629.8	0.33	637.2	0.29
Chromatogram of mobile phase change 66+34 ml	25	737.84	0.38	607.70	0.27

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

LOD is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions. LOQ is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions **Table 8.**

Table 8: Studies for AMLO and BISO

Parameters	Drug	
	Amlodipine	**Bisoprolol**
S.D.	3.3	01.05
SLOPE	21.94	19.23
LOD	0.040	0.1230
LOQ	0.01230	0.5460

DISCUSSION

The proposed methods for simultaneous estimation of **AMLO** and BISO in tablet dosage forms were found to be simple, accurate, economical and rapid. The method was validated as per the ICH Q2 (R1) guidelines. Standard calibration yielded correlation coefficient (r^2) 0.999 for both **AMLO** and BISO at all the selected wavelengths. The values of % RSD are within the prescribed limit of 2 %, showing high precision of methods and recovery was close to 100% for both drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed method is suitable for their simultaneous determination with virtually no interference of any additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully for simultaneous estimation of **AMLO** and BISO in formulations.

CONCLUSION

The developed HPLC methods in that linearity, precision, range, robustness were found to be more accurate, precise and reproducible. The methods were found to be simple & time saving. All proposed methods could be applied for routine analysis in quality control laboratories.

CONFLICT OF INTEREST

Authors have no conflicts of interest to declare.

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ABBREVIATION USED: HPLC: High performance liquid chromatography; **UV:** Ultraviolet; **ICH:** International Conference on Harmonization; **LOQ:** Limit of quantitation; **LOD:** Limit of detection; **RSD:** Relative standard deviation; **RT:** Retention time; **OPA:** Orthophosphoric acid; **AMLO:** Amlodipine; **BISO:** Bisoprolol; **FDA:** Food and Drug Administration; **SD:** Standard deviation.

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