Research Article

Analysis of Bisoprolol Fumarate and Amlodipine Besylate in Tablet Dosage Form by Using HPTLC

Nilam B Jadhav[1], Savita S Yadav[2]*

[1]Department of Quality Assurance Techniques, Bharati Vidyapeeth (Deemed to be University), Poona College of Pharmacy, Erandwane, Pune-411 038, India.

[2]Department of Pharmaceutical Chemistry, Bharati Vidyapeeth (Deemed to be University), Poona College of Pharmacy, Erandwane, Pune-411 038, India.

***Corresponding author's E-mail:** savitang2729@yahoo.com

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ABSTRACT

Antihypertensive fixed dose combination drugs are currently considered the most widely used in the treatment of arterial hypertension and chronic obstructive bronchitis. Combination of beta-blocker and long-acting dihydropyridine calcium channel blocker amlodipine besylate is recommended for the treatment of arterial hypertension. For low rate of bronchial complications, beta-blockers act on beta-1 adrenergic receptors. In view of the above concepts and considering the need, the objective of the study was to develop and validate a simple, fast, precise, selective and accurate HPTLC method for the simultaneous determination of Bisoprolol fumarate and Amlodipine besylate from tablet formulation. Chromatographic separation was achieved on precoated silica gel HPTLC aluminium plate 60 F254 using chloroform: ethanol: glacial acetic acid 2: 8: 0.1 (v/v/v) as mobile phase. Quantitative detection was observed by densitometric scanning at 231 nm. The Rf of Amlodipine besylate and Bisoprolol fumarate were 0.53 and 0.72, respectively. According to the ICH guidelines the method was validated with respect to specificity, linearity, accuracy, precision and robustness. Linearity was observed over the concentration range of 200-1200 ng/spot for Amlodipine besylate and Bisoprolol fumarate, respectively. The limits of detection and quantitation were found to be 40 ng/spot and 120 ng/spot for Amlodipine besylate and 50 ng/spot and 100 ng/spot for Bisoprolol fumarate. Mean percent recoveries obtained for both the drugs were 99.20 ± 0.41% for Amlodipine besylate and 99.11 ± 0.13 % for Bisoprolol fumarate. The method developed can be used for the routine analysis of Bisoprolol fumarate and Amlodipine besylate from their combined dosage form.

Keywords: Bisoprolol fumarate, Amlodipine besylate, HPTLC, Validation.

INTRODUCTION

Antihypertensive fixed dose combination drugs are currently considered the most widely used in the treatment of arterial hypertension and chronic obstructive bronchitis. Combination of beta-blocker Bisoprolol fumarate (BSF) and long-acting dihydropyridine calcium channel blocker Amlodipine besylate (AMLO) is recommended for the treatment of arterial hypertension. For exhibiting the low rate of bronchial complications, cardioselective beta-blockers act accurately on beta-1 adrenergic receptors. In view of the above concepts and considering the need, the objective of the study was to develop and validate a simple, fast, precise, selective and accurate HPTLC method for the simultaneous determination of Bisoprolol fumarate and Amlodipine besylate from tablet formulations.

Bisoprolol fumarate is (±)-1- [4- [[2-(1 methylethoxy) ethoxy] methyl] phenoxyl-3- [(1-methylethyl) amino]-2-propanol (E)-2-butenedioate (2:1), Bisoprolol fumarate is a synthetic beta – selective, cardioselective adreno receptor blocking agent. Chemically, AMLO is 3-ethyl-5- methyl (±)-2-[(2-aminoethoxy) methyl]-4-(2- chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridine dicarboxylate, monobenzenesulphonate, it is a long-acting calcium channel blocker, having utility in certain cardiovascular diseases like angina pectoris, and hypertension [1-3]. Chemical structure of both the drugs given in Fig. 1a and Fig. 1b.

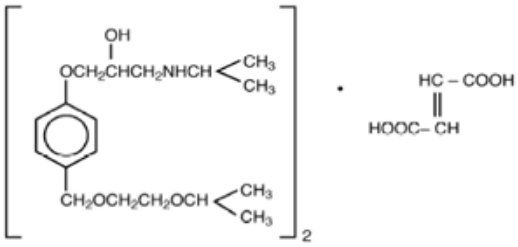

Figure 1a: Chemical structure of BSF

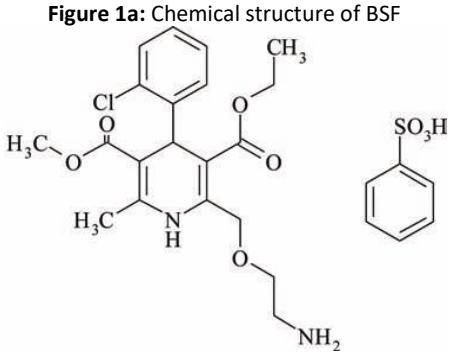

Figure 1b: Chemical structure of AMLO

Literature surveys acknowledge that various analytical methods are available for determination of BSF and AMLO individually as well as in combination with other drugs in pharmaceutical formulation and in biological samples. Such methods are Spectrophotometry [4-8], separation techniques like HPLC [9-11], LC-MS [12-13] and HPTLC [14-16]. There is no HPTLC method reported in literature that analyzes BSF and AMLO simultaneously in tablet

dosage form. Hence, the objective of this analysis was to develop and validate a HPTLC method that analyzes BSF and AMLO in tablet dosage form.

MATERIALS AND METHODS

Bisoprolol fumarate and Amlodipine besylate were supplied, as a gift sample, by Intas Pharmaceuticals Ltd, Ahmedabad and Cipla Ltd Goa, India. Concor® AM 5 tablet containing 5 mg of Bisoprolol fumarate and 5 mg of Amlodipine besylate were obtained commercially. AR grade chemicals and reagent were used for analysis and were purchased from Merck Chemicals, Mumbai, India.

Chromatographic separation was performed on aluminium foil plates coated with 0.2-mm layers of silica gel 60F254 (Merck). Samples were applied to the plates, as 6-mm bands, by means of a Camag Linomat 5 semi-automatic sample applicator (Camag, Muttenz, Switzerland) used at a constant application rate of 0.1 µL/s. Plates were developed with chloroform: ethanol: glacial acetic acid 2: 8: 0.1 (v/v/v) as mobile phase in a Camag twin-trough chamber previously saturated with mobile phase vapour for 20 min at room temperature. The development distance was approximately 8cm. After development the plates were scanned in absorbance mode at 231 nm by use of a Camag TLC Scanner 3 controlled by winCATS software. The slit dimensions were 5 mm × 0.45 mm and the source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range 190– 400 nm.

Preparation of standard solutions

A standard mixed stock solution was prepared by accurately weighing 20 mg of Bisoprolol fumarate and 20 mg of Amlodipine besylate into a 10 mL volumetric flask. The drugs were dissolved in methanol and the solution was diluted to volume to get the concentration 2000 µg/ mL each of Bisoprolol Fumarate and Amlodipine.

Analysis of marketed formulation

Twenty tablets (Concor® AM 5) were selected, each containing 5 mg of BSF and 5 mg of AMLO, weighed and finely powdered. Powder equivalent to 5 mg of BSF and 5 mg of AMLO was transferred into 50 mL volumetric flask and extracted with methanol. The solution was sonicated for 20 min and filtration through whatman filter paper no. 41 and residue was washed with methanol. Required further dilutions were made with methanol.

Method validation [17-18]

Analytical method performance parameters such as specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and robustness were validated as per ICH guidelines.

RESULTS AND DISCUSSION

Method optimization

The method was optimized with a thought to develop HPTLC method for simultaneous determination of BSF and AMLO. The method was optimized through the trials of several mobile phases until good resolution obtained between two drugs. Chloroform: ethanol: glacial acetic acid 2: 8: 0.1 (v/v/v) was finally considered as the mobile phase which resulted in sharp, well-defined peaks with a good resolution.

Specificity

To evaluate the specificity of the method by analyzing standard drugs and samples extracted from formulations. The band for BSF and AMLO was confirmed by comparing the Rf of the samples with those of the standards. The method was specific for BSF and AMLO, since it resolved the peak of AMLO (Rf = 0.53) and BSF (Rf= 0.72) in presence of other excipients in the formulation (Fig. 2).

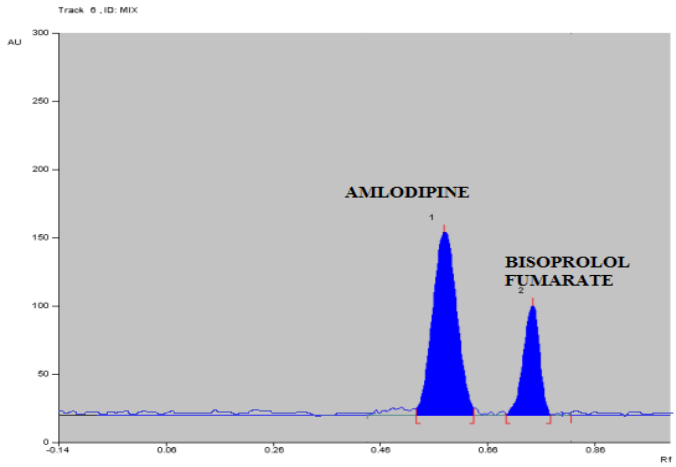

Figure 2: Densitogram of AMLO (peak 1) and BSF (peak 2)

Method validation

The following parameters as per ICH guidelines were validated for HPTLC method:

Linearity and range

Six different concentrations 0.1 to 0.6 µL from the standard solution were spotted on the TLC plate. Each concentration was applied in replicates on the TLC plate and using the previously described mobile phase the plate was then developed. The peak areas obtained were plotted against the concentration range of 200-1200 ng/spot for both AMLO and BSF, respectively to obtain the calibration curves and correlation regression equations were calculated. The linear regression equations were found to be Y = 4.8269x + 408.91 (r^2 = 0.9997) for AMLO and Y= 5.7092x + 1048.2 (r^2 = 0.9992) for BSF as shown in Fig. 3.

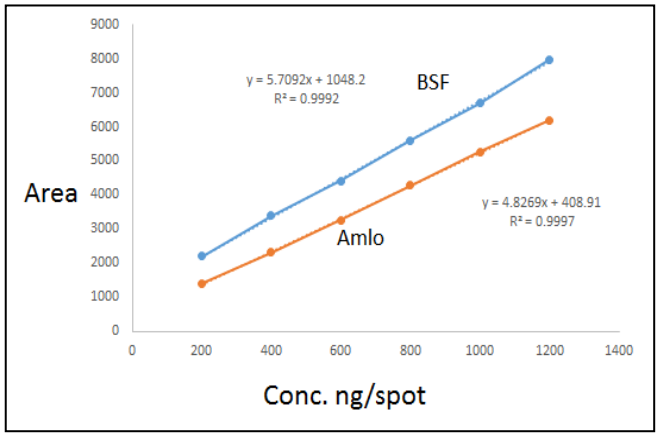

Figure 3: Calibration curve for AMLO and BSF

Limit of detection and limit of quantitation

To assess the limits of detection (LOD) and quantification (LOQ) solutions of lower concentration in the linear range of the calibration plot were used. From the slope (S) of the calibration plot and the standard deviation (SD) of the response LOD and

LOQ were calculated using the equations, LOD = 3.3 × SD/S and LOQ = 10 × SD/S. 40 ng/spot and 120 ng/spot for AMLO and 50 ng/spot and 100 ng/spot for BSF were found as value of LOD and LOQ, respectively.

Precision

Precision was determined by taking three replicates of concentration 400, 800, 1200 ng/spot and performed intraday (within a day) and interday (day to day) studies over duration of a week. Less than 2 % relative standard deviation was found for both intraday precision and inter-day precision studies as shown in Table 1.

Robustness

For checking reliability of the method, robustness of analytical procedures was carried out by making small changes in the chromatographic conditions like change in mobile phase composition (± 0.1 mL), development distance (± 0.5 cm) and duration of saturation (± 5 min.) and finding the effects on the results obtained. The % RSD shown in Table 2 and it was found to be less than 2% indicates the robustness of the method.

Accuracy studies

Checking the closeness of conformity between the values which is accepted as a conventional true value and the value found using accuracy studies for both AMLO and BSF by standard addition method at three different concentration levels 80%, 100%, and 120% within range of linearity for drugs. The recovery found was listed in Table 3.

Table 1: Precision studies of AMLO and BSF

Conc. (ng/spot)	Intra-day precision (n=3)		Inter-day precision (n=3)	
	Measured Avg. area ±SD	(%) RSD	Measured Avg. area ±SD	(%) RSD
AMLO				
400	4194.33 ± 3.67	0.09	4197.67 ± 2.11	0.05
800	7238.13 ± 6.73	0.10	7234.8 ± 0.96	0.013
1200	8988.3 ± 6.32	0.07	8991.63 ± 1.10	0.012
BSF				
400	2818.87 ± 2.92	0.10	2822.2 ± 2.86	0.101
800	4683.67 ± 5.38	0.12	4687.33 ± 0.21	0.004
1200	4887.47 ± 6.21	0.13	4890.8 ± 0.46	0.009

Table 2: Robustness study of AMLO and BSF

Conditions	AMLO			BSF		
	Rf	Avg. Area	% RSD	Rf	Avg. Area	% RSD
Mobile phase composition ± 0.1 mL (v/v/v)						
Chloroform: ethanol: glacial acetic acid 1.9: 7.9: 0.1	0.52	4091.3	0.17	0.73	2510.3	0.01
Chloroform: ethanol: glacial acetic acid 2: 8: 0.1	**0.53**	**4191.6**	**0.19**	**0.72**	**2820.7**	**0.05**
Chloroform: ethanol: glacial acetic acid 2.1: 8.1: 0.1	0.53	4258.3	1.54	0.78	2837.4	0.54
Development distance (± 0.5 cm)						
7.5 cm	0.51	4224.6	1.45	0.72	2850.7	1.06
8.0 cm	**0.53**	**4258.3**	**1.54**	**0.72**	**2837.4**	**0.54**
8.5 cm	0.56	4244.9	1.36	0.78	2847.4	1.08
Duration of saturation (± 5 min.)						
15 min.	0.52	4254.9	1.48	0.73	2834.0	0.41
20 min.	**0.53**	**4259.3**	**1.57**	**0.72**	**2924.5**	**0.43**
25 min.	0.53	4254.9	1.58	0.72	2830.7	0.62

Table 3: Accuracy study of AMLO and BSF

Label claim (mg/tablet)	Amount Added (%)	Total amount (mg)	Amount recovered (mg)	(%) Recovery	Mean (%) Recovery ± SD
AMLO (5 mg)	80	9	8.888	99.41	99.20 % ± 0.41
	100	10	9.877	99.47	
	120	11	10.937	98.73	
BSF (5 mg)	80	9	8.831	99.15	99.11 % ± 0.13
	100	10	9.911	99.21	
	120	11	10.93	98.96	

Analysis of tablet formulation

Concor® AM 5 tablets were analysed, sharp and well-defined peaks for AMLO and BSF were obtained at Rf 0.53 ± 0.02 and 0.72 ± 0.03, respectively, when scanned at 231 nm. The percent content of the label claim calculated were 99.59 % for AMLO and 99.43 % for BSF.

CONCLUSION

HPTLC method has been developed and validated for simultaneous estimation of Bisoprolol fumarate and Amlodipine besylate in tablet dosage form. Based on the results, the stability and reliability of the method indicate that the evaluation of HPTLC method for both the drug is simple, precise, accurate, sensitive and robust. The proposed method can be employed for the routine quality control analysis laboratories.

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