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Original Research Article

Design of Optimized RP-HPLC Method for Quantitative Analysis of Bisoprolol Fumarate in Bulk and Pharmaceutical Dosage Form

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Abstract

In this study an effective method was developed to assay Bisoprolol fumarate in tablets dosage form. The chromatographic separation was achieved on Reprosil pure basic C18 analytical column. A mixture of acetonitrile + Potassium dihydrogen phosphate buffer (0.050 mol L-1) (30:70 V/V), pH 3.5 was used as the mobile phase, effluent flow rate monitored at 1.0 mL/min, and UV detection at 233 nm. In forced degradation studies, the effects of acid, base, oxidation, UV light and temperature which were investigated showed no interference in the peak of drug. The proposed method was validated in terms of specificity, linearity, robustness, precision and accuracy. The method was linear at concentrations ranging from 5µg/mL to 17.5µmg/mL, precise (intra- and inter-day relative standard deviations R.S.D. < 2 %), (r^2 = 0.9995).

Keywords: DOE, Validation, RP-HPLC, Bisoprolol Fumarate.

INTRODUCTION

Bisoprolol is a cardio selective beta-blocker. It is given as the fumarate in the management of hypertensive and angina pectoris. Chemically, it is 1-(propan-2-ylamino)-3-[4-(2-propan-2-yloxyethoxymethyl) phenoxy] propan-2-ol. Figure 1.

Bisoprolol is a drug belonging to beta blocker drugs used primarily for the treatment of cardiovascular diseases. Various methods for determination of bisoprolol by fluorimeter, HPLC and densitometry are reported in literature. Also HPTLC, HPLC and spectrophotometric methods are reported for determination of Bisoprolol fumarate alone or in combination with other drugs.

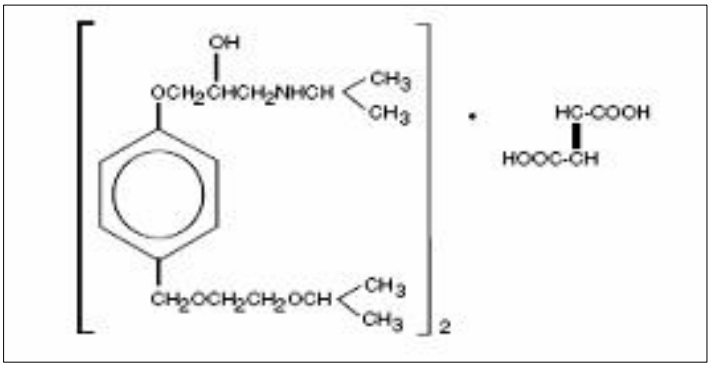

Figure 1: Chemical structure of bisoprolol fumarate

The aim of this method is being to develop and validate a simple, precise and accurate chromatographic method for the estimation and quantification of bisoprolol fumarate in bulk material and in tablets. Further, this study is designed to validate the developed method as per ICH guidelines [1-3].

MATERIALS

Bisoprolol fumarate was a gift sample from Aurobindo Pharma India Ltd. All chemicals and reagents used were of analytical grade and purchased from SDFCL SD fine Chem limited India.

Instruments

SHIMADZU HPLC Model LC 20230C 3D Prominence I, Pump LC 2030, Detector LC 2030/4040 PAD and software post run analysis lab solution version 5.81, data acquisition was carried out with lab solution software, the data acquisition rate 1.564 Hz., column C18 (250x4.6 mm.5µm). All weights were taken on electronic balance (Sartorius CAP 224S Germany).

Optimization of Chromatographic Conditions

The chromatographic method development for the determination of bisoprolol fumarate was optimized by applying factorial design approach.

Table 1: Factors level

Factor	Level	
	+1	-1
Acetonitrile % X1	70	60
Buffer V/V X2	40	30
Flow rate mL/min X3	1.2	1.0

Table 2: Chromatographic condition as a factorial design 2^3

Run	Factors			Response		
	X1	X2	X3	RT*	Tf*	Peak area
1	30	60	1.0	4.016	1.08	1350.81
2	40	60	1.0	2.650	1.25	1462.39
3	30	70	1.0	5.816	1.06	1486.61
4	40	70	1.0	3.133	1.22	1546.933
5	30	60	1.2	3.283	1.30	1396.2535
6	40	60	1.2	2.300	1.43	1540.88
7	30	70	1.2	4.500	1.25	1486.730
8	40	70	1.2	2.750	1.38	1511.338

RT: retention time, **Tf**: tailing factor

By Applying statistical software SPSS 23, the model was fit for analysis of Bisoprolol Fumarate where r^2 was 0.986, the tailing factor was highly significant, and the standard deviation 0.015, the experimental value of tailing factor was 1.08 min and the predictor value 1.051 with predicting error about 2.8%.

According to optimization of the analysis the mobile phase was selected as CAN 30:70 Buffer pH 3.5 which indicated that increasing of phosphate buffer concentration improve the tailing factor.

Preparation of Stock Standard Solution and Selection of Wavelengths

A stock standard solution was prepared by dissolving 100 mg of Bisoprolol Fumarate in a 100 ml of 50 % v/ v methanol to obtain a concentration of 1000 µg/ml. Appropriate concentration of 10 µ g/ml was prepared and scanned in the UV-visible over the range 400–200 nm, the first derivative was recorded.

Chromatographic Conditions

Chromatographic separation was achieved at ambient temperature and the detection was carried at 233 nm at a flow rate of 1 mL/min. Run time was kept at 20 min. Prior to the injection of drug solution, column was equilibrated for 60 min with the mobile phase flowing through the system. The injection volume was 20 µL for assay level. The analysis has been performed by using Reprosil pure basic C18 (250 × 4.6 mm.5 um) and the mobile phase containing acetonitrile: phosphate buffer (30:70) at pH 3.5 (adjusted with phosphoric ac.

Validation of the method [4, 5]

Study of Linearity Curves

For bisoprolol fumarate, linearity was observed by diluting appropriate aliquots of the working standard stock solution 5µg/mL, 7.5, 10, 12.5, 15, and 17µg/ mL into a series of 50-mL volumetric flasks with 50% methanol and plotted concentration against peak area to obtain the calibration graph. The statistical parameters of the calibration curve, such as the correlation coefficient, regression equation, limit of detection, and limit of quantitation, for bisoprolol fumarate were calculated.

Recovery Studies

To the pre-analyzed sample solutions, a known amount of the stock standard solution was added at different levels, i.e. 80%, 100%, and 120%. The solutions were re-analyzed by the proposed method.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under prescribed conditions. Repeatability of the method was checked by injecting replicate injections of 5 and 10 µg/ml of the solution for 6 times on the same day as intra-day precision study of

Bisoprolol and the chromatogram was recorded. The mean area and % relative standard deviation (RSD) was calculated. From the data obtained.

Repeatability

Repeatability was determined by analyzing 10 µg/ml concentration of bisoprolol fumarate solution for six times.

Sensitivity

The sensitivity of measurements of bisoprolol fumarate by the use of the proposed method was estimated in terms of the limit of quantification (LOQ) and limit of detection (LOD). The LOQ and LOD were calculated using equation LOD = 3.3 × *N/B* and LOQ = 10 × *N/B*, where '*N*' is standard deviation of the absorbance of the drugs (n = 3), taken as a measure of noise, and '*B*' is the slope of the corresponding calibration curve.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was observed that the variations like flow rate of the mobile phase, column temperature, ratio of organic content in the mobile phase, etc. Does not have any significant effect on the method performance, which demonstrated that the developed RP-HPLC method is robust.

The proposed method was determined for 2.5 and 5mg of dosage form of Bisoprolol Fumarate by analysis of aliquots from a homogenous sample by two analysts using the same operational and environmental conditions for the proposed method.

Analysis of Marketing, Formulation

Twenty tablets of each different brand were accurately weighed, average weight determined and ground into fine powdered. A quantity of powder equivalent to 5mg of tablets was transferred into a 100-ml volumetric flask containing 30 ml of 50% *v/v* methanol, sonicated for 15 min; the volume was adjusted to the mark using the same solvent and filtered through Sartorius filter paper grade 292. An appropriate volume 10 ml was transferred into a 25-ml volumetric flask and the volume were adjusted to the mark to obtain the desired concentration of 10 µg/ml for 2.5 mg of tablet, and 5 ml was transferred into 25 volumetric flasks to obtain 10µg for 5 mg of tablet.

Standard Solution

From the stock solution, 0.5ml was pipetted out in 50 mL volumetric flask to get a concentration of 10 ug/ml.

Acid and Base Degradation

Accurately weight of 100mg of Bisoprolol Fumarate was transferred into 100 volumetric flasks. 10ml of methanol was added and sonicated for 15 minutes with intermittent shaking; 5 ml of 0.1M of HCl or of 0.1M NaOH was added separately. The sample was heated in boiling water bath for 45 minutes, cool to room temperature and diluted to volume with diluent. The sample was neutralized to pH 7 by adding 0.1M HCl or 0.1 M NaOH, mixed well. The acidic degradation and the alkaline forced degradation were performed in the dark in order to exclude the possible degradation effect of light. This solution was filtered through 0.45um filter, 5ml of the filtrate was transferred to 25 ml volumetric flask, diluted to the volume with the mobile phase and injected into the HPLC system.

Oxidative Degradation

Accurately weight of 100mg of Bisoprolol fumarate was transferred into 100 volumetric flasks. 10ml of methanol was added and sonicated for 15 minutes with intermittent shaking. 5 ml of 3% H_2O_2 was added. The sample was heated on a boiling water bath for 45 minutes, cool to room temperature and diluted to volume with diluent, mixed well. This solution was filtered through 0.45um filter, 5ml of the filtrate was transferred to 25 ml volumetric flask, diluted to the volume with the mobile phase and injected into the HPLC system.

Thermal Degradation

Accurately weight 100mg of Bisoprolol fumarate was transferred into 100 volumetric flasks. 10ml of methanol was added and sonicated for 15 minutes with intermittent shaking. The sample was heated on a boiling water bath for 45 minutes, cool to room temperature and diluted to volume with diluent, mixed well. This solution was filtered through 0.45um filter, 5ml of the filtrate was transferred to 25 ml volumetric flask, diluted to the volume with the mobile phase and injected into the HPLC system.

RESULTS AND DISCUSSION

Design of experiments is part of the statistical tools which can be used to facilitate learning of the connections between processing and products, and facilitate improvement activities [4]. Quality improvement is then connected both with a deeper understanding of the product itself but also to the factors defining the process., the role of designed experiments in quality improvement by the use of sequential experimentation. One of the ideas behind sequential experimentation is that the benefits of controlling the conditions and the direction of inquiry outweigh the costs when compared to the strategy of using historical data, so the experimental design was the process of choosing how to run an experiment.

The purpose of DOE is to determine how a response Y depends on one or more input variables or predictors X so that future values of the response can be predicted from the input variables [6, 7].

In statistics, a full factorial experiment is an experiment whose design consists of two or more factors, each with discrete possible values or "levels", and whose experimental units take on all possible combinations of these levels across all such factors.

The aim of this study is to develop a simple, precise and accurate revese phase HPLC methodfor the determination of atorvastatin calcium in pharmaceutical dosafe form as per ICH guidelines [8].

In this work, three factors with two level were applied to predict the retention behavior of Bisoprolol Fumarate and optimize their isocratic elution using acetonitrile as organic modifier and buffer as mobile phase.

The process of choosing how to run an experiment and measuring the change by manipulation independent factor in X -axis with respect to response in Y-axis this is factorial design an eight- run, 3^2 factorial design of three factors at two level was set up to standardize the spectrographic condition which is likely to be employed. Percentage of acetonitrile in the organic phase (X1), a proportion of phosphate buffer pH 3.5 % (X2) and flow rate (X3) as per 3^2 factorial design are represented in the Table1: Factors and their corresponding levels as per 3^2 factorial design. The optimization of the method was done by selected of suitable solvents such as methanol and acetonitrile, different columns C8, C18, detection wavelength and analyte concentration. The detection wavelength of 233nm was selected after scanning the standard.

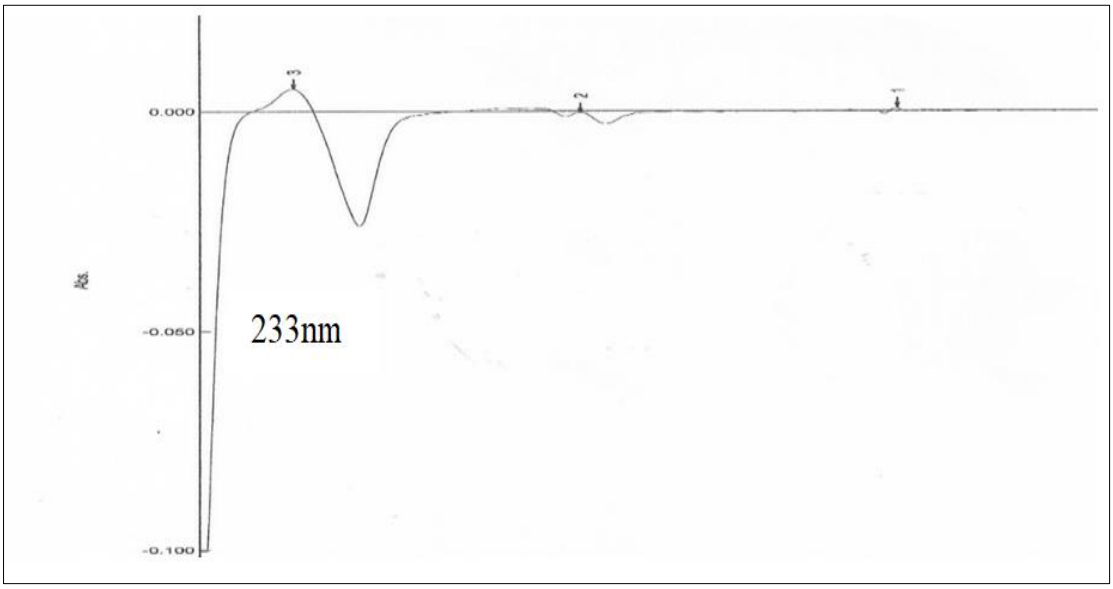

Figure 2: First derivative absorption spectrum of bisoprolol fumarate working standard

The method was validated according to ICH Q2B R1 guidelines for the validation of analytical procedure in order to determine the linearity, sensitivity, precision, accuracy and ruggedness.

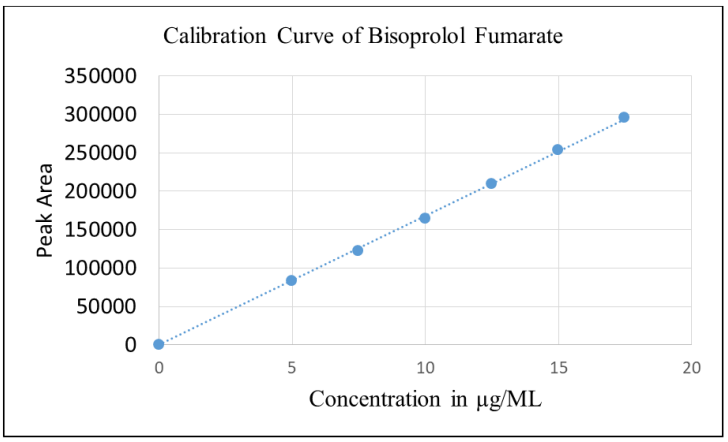

Figure 3: Calibration curve of bisoprolol fumarate

Table 3: System suitability Parameters for Bisoprolol Fumarate by Proposed method

Name of compound	Retention time	Theoretical plate	USP Tailing	Resolution	S/N
Bisoprolol	6.236	3906	1.055	14.42	16.28

Recovery is expressed as the amount / weight of the compound of interest analyzed as a percentage to the theoretical amount present in the medium.

Table 4: Results of recovery study test for bisoprolol fumarate

Level of addition %	Drug	Tablet strength (n=3) µg/ml	The amount added (n=3) µg/ml	Average amount recovery	Recovery
80	Bisoprolol	5.0	4.0	3.98	99.50
100	Bisoprolol	5.0	5.0	5.01	100.2
120	Bisoprolol	5.0	6.0	5.97	99.50
Average					99.75
RSD %					0.405

The precision of the assay of bisoprolol fumarate was performed by repeatability (intra-day) and intermediate precision the concentration used 10µg/ml and reported as RSD%.

Table 5: Repeatability of the validated method Concentration 10µg/mL

Sample no	Response peak area at 233nm	Retention time	USP Tailing
1	85433	6.252	1.058
2	85403	6.249	1.058
3	85426	6.252	1.056
4	84609	6.242	1.058
5	85023	6.243	1.056
6	85063	6.263	1.056
Mean	85159	6.250	1.057
%RSD	0.384	0.12	0.10
Limit	NMT2 %	NMT1%	T ≤ 2

Table 6: Reproducibility of the validated method Concentration 5µg/mL

Run	Retention time	Peak area at 233nm	USP Tailing
1	6.136	83046	1.053
2	6.236	82979	1.055
3	6.248	82424	1.055
4	6.236	82979	1.047
5	6.239	82633	1.054
6	6.214	82466	1.071
Mean	6.217	82754	1.055
% RSD	0.71	0.33	0.75
Limit	NMT1%	NMT2%	T ≤ 2

Table 7: Inter-day intermediate precision of the validated method

Repeatability precision		Run	Peak area at 233 nm	Assay	% RSD N =6
Sample	Concentration				
Bisoprolol fumarate	10µg/ml	1	85443	100.35	0.45
		2	85423	100.33	
		3	84493	99.24	
		4	85453	100.37	
		5	85123	99.97	
		6	84911	99.73	

Table 8: Intermediate precision of the validated method

Concentration	Analyst 1	Analyst 2
10µg/ml	84334	86014
	84567	85447
	85224	85054
	85014	83025
	85114	85070
	85325	84992
Average	84299	84933
SD	358.31	922.86
%RSD	0.425	1.086

Table 9: Assay of bisoprolol fumarate in tablet formulation

Drug	Label claim mg/tablet	Amount found*	Recover	% RSD
Amicor	2.5	2.506	100.24	1.00
Bisocard	5.0	4.95	99.00	1.30
Amicor	10	9.86	98.60	1.35

*Average of three estimation

Table 10: Robustness studies of Bisoprolol fumarate

Robust condition		Bisoprolol Fumarate	
		Peak area	USP tailing
Flow rate	0.9 mL/min	546623	1.086
	1.1mL/min	492883	1.071
Organic Composition	10% less	495341	1.080
	Actual	505561	1.07

Table 11: Robustness change pH of mobile phase

Robust condition		Bisoprolol Fumarate	
		Peak area	USP tailing
pH of mobile phase	pH 3.3	489470	1.064
	PH 3.5	48850	1.070
	PH 3.7	495341	1.080

Specificity

Spectral purities of atorvastatin chromatography peaks were evaluated for the interference of the tablet excipients, degradation components or due to the presence of impurities as per the methodology. In the work, a solution containing a mixture of the tablet excipients was prepared using the sample preparation procedure to evaluate possible interference peak. The representative chromatogram did not show any other peak, which confirms the specificity of the method.

Specificity experiment shows that there is no interference of excipients with the main peaks, which confirmed the specificity of the method.

For stress testing under different conditions, the major degradation under acidic condition up to 23.91% Table 3, under alkaline condition Bisoprolol was stable compared to peroxide degradation, 20.38 %. Bisoprolol fumarate more susceptible to oxidation and hydrolysis by acid, to some extend bisoprolol stable under alkaline hydrolysis and thermal degradation table 12.

Table 12: Degradation of stress testing of Bisoprolol Fumarate

S.NO	Condition	%Assay of Bisoprolol Fumarate	% degradation
1	No stress treatment (control sample)	99.81	Nil
2	Acid	75.9	23.91
3	Alkali	97.12	2.69
4	H_2O_2	79.43	20.38
6	Thermal	90.27	9.54

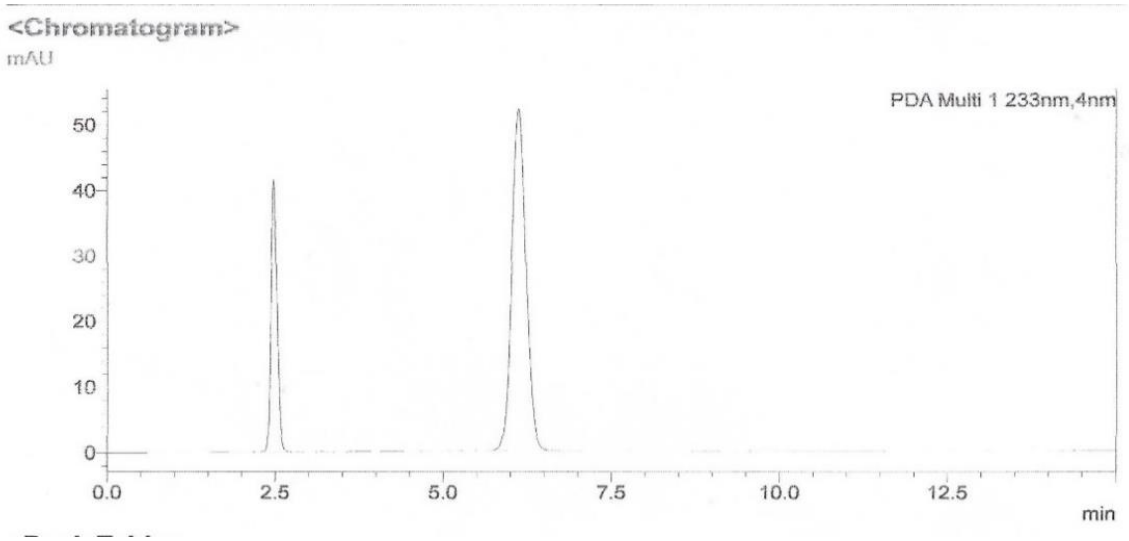

Figure 4: Validative chromatogram of Bisoprolol fumarate working

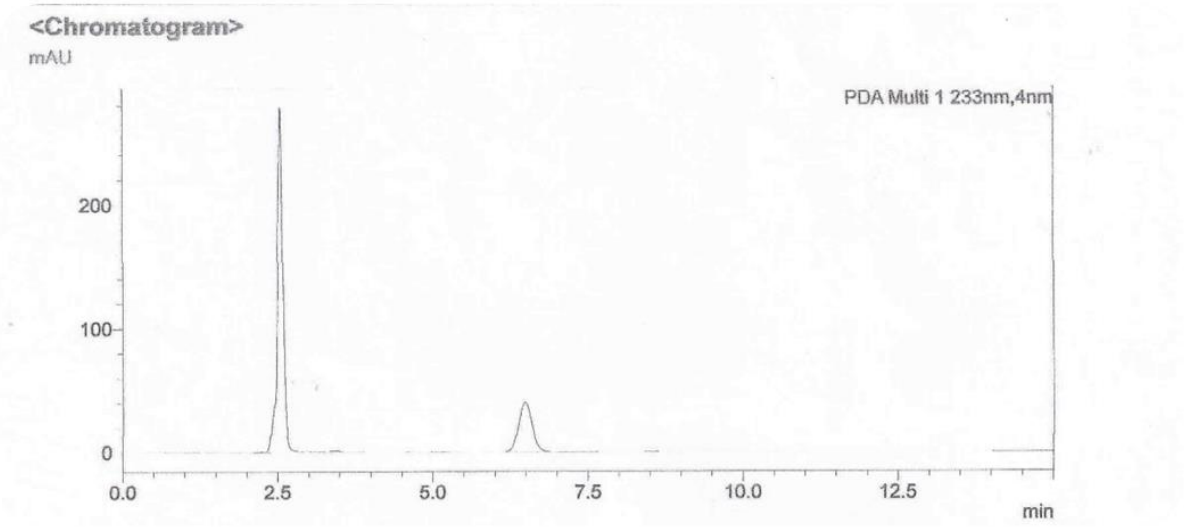

Figure 5: Chromatogram corresponding to BF solution subjected to Oxidation

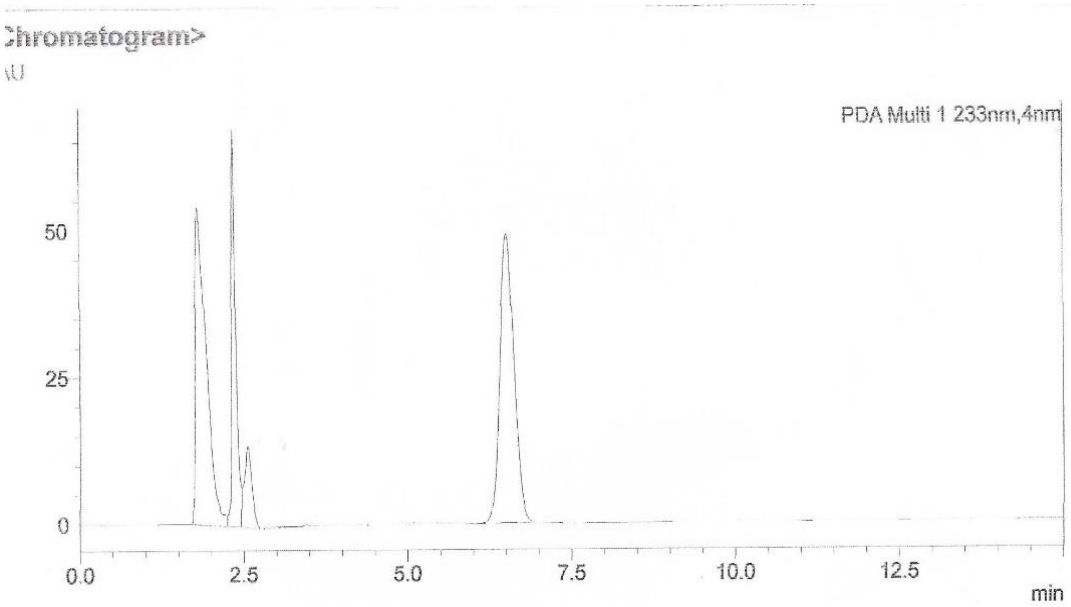

Figure 6: Chromatogram corresponding to BF solution subjected to Base hydrolysis

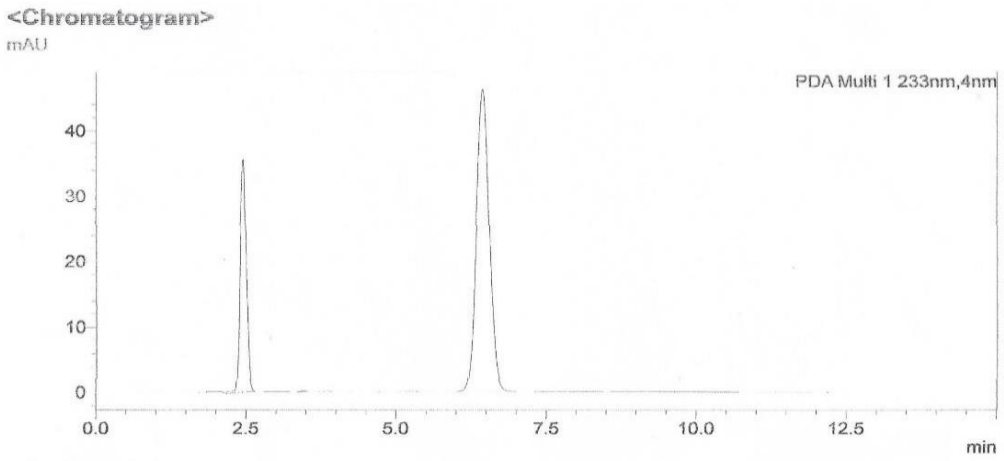

Figure 7: Chromatogram corresponding to BF solution subjected to thermal

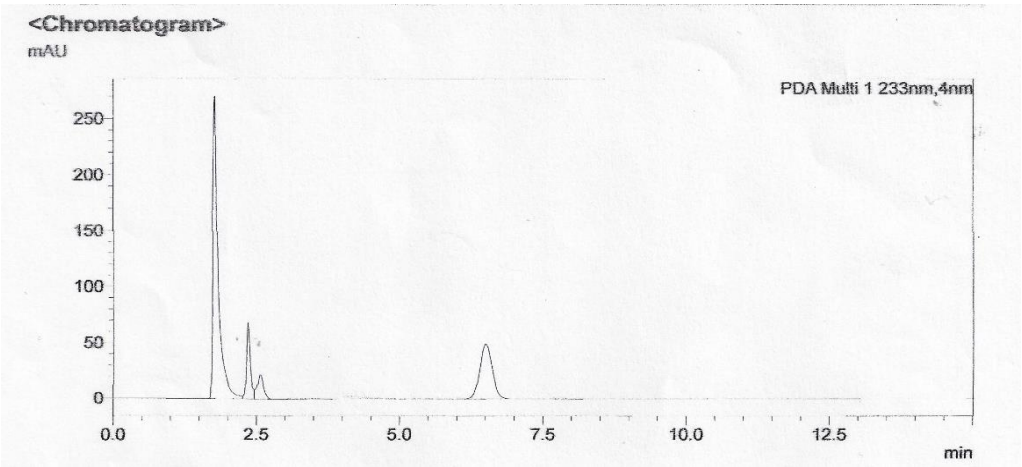

Figure 8: Chromatogram corresponding to BF solution subjected to Acid hydrolysis.

Detection and quantitation limit

The limit of detection (LOD) and limit of quantitation (LOQ) of atorvastatin was determined by using the signal to noise ratio approach as defined in ICH guidelines. According to the determined signal to noise ratio, the LOD and LOQ for atorvastatin was 0.41 µg/ml and 1.24 µg/ml, respectively.

The lowest values of LOD and LOQ, which obtained by the method, indicate the sensitivity of the method.

Assay of Tablet

Table 13: Assay of Bisoprolol fumarate tablet 5 mg

Drug	n	Amount claimed (mg/tablet)	The amount found (mg/tablet)	Recovery	RSD
Bisoprolol Fumarate	3	10	9.88	99.0	0.41

The assay results of the pharmaceutical formulation of this method are highly reproducible, reliable and are in good agreement with a label claim of the drug, thus this method can be useful for routine work to determine of atorvastatin calcium in a dosage form.

Table 14: Method validation parameters

Parameters	Results
Linear range	5 -17.5µg/ml
Regression equation	Y =16965x-2365
Correlation coefficient (r^2)	0.9995
Slope	16965
Intercept	2365
LOD µg/ml	0.41
LOQ µg/ml	1.24

The proposed method is highly sensitive for determination of bisoprolol fumarate in tablet dosage form and good recovery which indicate the suitability of the method.

CONCLUSION

It is evident from the results of validation that the method is accurate, sensitive, selective, and precise for derivative spectroscopic estimation of bisoprolol fumarate. Moreover, the method is economical, simple and rapid, hence can be employed for routine analysis in quality control laboratory for estimation of bisoprolol fumarate from marketed formulation and raw material.

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