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Research Article

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF METOPROLOL AND ATORVASTATIN BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN ITS BULK AND PHARMACEUTICAL TABLET DOSAGE FORM USING BIORELEVANT DISSOLUTION MEDIA (FASTED STATE SMALL INTESTINAL FLUID)

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ABSTRACT

Objective: A present investigation is based on method development and validation for the simultaneous determination of metoprolol and atorvastatin by reversed-phase high-performance liquid chromatography in its bulk and pharmaceutical dosage form using a biorelevant dissolution media (fasted state small intestinal fluid).

Methods: The chromatographic separation technique performed by an isocratic method for this column used Inertsil ODS-3 (4.6×150 mm, 5 µm). The ratio of mobile phase used is phosphate buffer 4.8 pH: acetonitrile (35:65v/v), flow rate 1 ml/min, and analysis time 15.0 min, UV detection was at 244 nm.

Results: According to the International Conference on Harmonisation Q2 (R1) guidelines, the method validation was done. Peaks were observed at 2.227 min and 5.819 min, concentration range of linearity was obtained at 50–250 µg/ml and 10–50 µg/ml, linearity correlation coefficients were 0.9997 and 0.9995, limit of detection was 0.33 mg/ml and 0.21 mg/ml, and limit of quantification was 1.08 mg/ml and 0.69 mg/ml for metoprolol and atorvastatin, respectively.

Conclusion: The obtained results for this method validation are within acceptance criteria. This method was more economical and stable for routine analysis.

Keywords: Metoprolol and atorvastatin, Reversed-phase high-performance liquid chromatography, Method development, Validation, International Conference on Harmonisation Q2 (R1), Biorelevant dissolution media (fasted state small intestinal fluid).

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INTRODUCTION

Metoprolol acts as a competitive β1-adrenergic receptor antagonist agent [1,2] (cardioselective) used as the antihypertensive agent. Antagonist activity of this agent is mainly because of more substituents present on the para position [3,4]. It shows membrane-stabilizing effects prescribed at a high dose than the dose required to show antagonist property [5]. The IUPAC name for metoprolol is 1-[4-(2-methoxyethyl] phenoxy]-3-(propan-2-ylamino)propan-2-ol. Chemical structure for metoprolol is shown in Fig. 1.

Atorvastatin is a statin and used as the lipid-lowering agent. It decreases the cholesterol levels by inhibiting the 3-hydroxy-3-methylglutaryl (HMG)-CoA enzyme because, in mevalonate pathway, it is a rate-determining enzyme in cholesterol. Atorvastatin primarily [6,7] acts on the liver and selectively inhibits the release of HMG-CoA reductase enzyme. HMG-CoA reductase is responsible for converting HMG-CoA to mevalonate in the cholesterol biosynthesis, and by this enzyme, conversion reaction prevents the synthesis of hepatic cholesterol [7-9]. It will encourage the hepatic uptake of cholesterol and decreases serum cholesterol levels by stimulation of hepatic low-density lipoprotein-cholesterol receptors [10]. The IUPAC name for atorvastatin is (3R, 5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid. Chemical structure for Atorvastatin is shown in Fig. 2.

From literature review [11-26], we found that there were no methods available for simultaneous determination of metoprolol and atorvastatin in a combined dosage form by reversed-phase high-performance liquid chromatography (RP-HPLC) using biorelevant dissolution media. This research work denotes a novel, economical, accurate, precise, specific, robust, rugged RP-HPLC method developed in the selected solvent system (mobile phase) in biorelevant dissolution media (fasted state small intestinal fluid [FaSSIF]) [27-31], and the validation was performed as per the International Conference on Harmonisation (ICH) Q2 (R1) guidelines [32].

MATERIALS AND METHODS

Reagents and chemicals

The metoprolol and atorvastatin pure standards were supplied by Syncorp Clincare Pvt. Ltd., Dilsuknagar, Hyderabad. The marketed formulation tablets labeled to contain 50 mg of metoprolol and 10 mg of atorvastatin, manufactured by Emcure Pharmaceuticals Ltd. (Metpure St), were obtained from the market. Analytical reagent grade and HPLC Grade chemicals procured from SD Fine-Chem Ltd., Mumbai (Mumbai, India) were used in the research.

Instruments used

The instrument was used Waters HPLC (717 series), Inertsil ODS-3 column, UV detector, data handling system EMPOWER2 software, UV-Visible double beam spectrophotometer (Labindia), analytical balance 0.1mg sensitivity (SHIMADZU), pH meter (Labindia), and ultrasonicator.

Blank FaSSIF

Weigh and dissolve NaOH (1.74 g), NaH_2PO_4 (19.77 g), and NaCl (30.93 g) in 5 L of HPLC grade distilled, and the pH was adjusted to 6.5 by using 1N HCl.

Diluent
Weigh and dissolve 3.3 g sodium taurocholate in approximately 500 ml of blank FaSSIF. Then, add 11.8 ml of methylene chloride solution containing 100 mg/ml of lecithin, and it produces an emulsion which is turbid. This solution was subjected to vacuum at a temperature of about 40°C under pressure at 500 mbar for 10 min and followed by 30 min at 10 mbar to get a clear solution. After that the solution was cooled to 27°C and make up the volume to 2 L with blank FaSSIF.

Stock solutions
Weigh and transfer pure 10 mg of metoprolol and atorvastatin separately into 10 ml volumetric flasks. Then, add 7 ml of diluent and ultrasonicated for 15 min. Filter the solution using membrane filter paper (0.45 µm), and volume make up to 10 ml using the same diluent. Five levels of linearity concentrations were prepared by mixed appropriately and further diluted to get 50–250 µg/ml of metoprolol and 10–50 µg/ml of atorvastatin. Inject the series concentrations in triplicate into the column, and the average peak areas are recorded from chromatograms. Linearity graph was plotted peak area against concentrations.

Mixed working solution
To prepare separately 1 mg/ml of metoprolol and atorvastatin solution by using stock solution. From this above solutions, pipette out 1 ml of metoprolol solution and 0.1 ml of atorvastatin solution into a 10 ml volumetric flask and make up the volume with a diluent, to get concentrations 100 µg/ml and 10 µg/ml of metoprolol and atorvastatin working solutions, respectively.

Test solution
According to I.P method, take twenty tablets and weighed. Then, the tablets are triturated in a mortar to get smooth powder. The amount of drug present in a powder which is equivalence to standard drug of 10 mg of atorvastatin. The powder was transferred to a 100 ml of volumetric flask and add approximately 70 ml of diluent, and the resulted solution was subjected to sonication for 15 min by using ultrasonicator, Then, the solution was filtered using membrane filter paper (0.45 µm) and volume make up to 100 ml using the same diluent. From this, take 1 ml and transfer to six 10 ml volumetric flasks, and then, the volume was made up mark with the diluent. These solutions are injected 3 times each sample solution into the column and the results are mentioned as a function of mean of all replicas.

Study of spectra and selection of wavelength
Working standard solutions were scanned an entire range of UV in a 1 cm cell against blank using UV-spectrophotometer. The absorption maxima of metoprolol and atorvastatin were selected from spectral data, and isosbestic wavelength was selected from overlain spectra of UV spectrophotometer. An isosbestic point was found to be at 244 nm. The UV spectrum of metoprolol and atorvastatin is shown in Fig. 3.

Optimization of HPLC method
The column used in this method was performed on Inertsil ODS-3(4.6×150 mm, 5 µm). The method was optimized with a mobile phase as its composition phosphate buffer 4.8 pH and acetonitrile (35:65v/v) that run isocratically; conditions were optimized with the rate at which mobile phase runs at 1.0 ml/min, UV detection at 244 nm, and analysis time was 15.0 min.

Validation of method
This method was validated according to the ICH Q2 (R1) guidelines. The validation parameters performed were system suitability, linearity range, accuracy data, precision (intra and inter), limit of detection (LOD), limits of quantification (LOQ), and robustness.

Forced degradation studies
Active pharmaceutical ingredients of metoprolol and atorvastatin were subjected to keep in degradation ways and find the extent of degradation of a product by this method. The parameters were carried out for forced degradation studies are acid, base, peroxide, thermal and photo degradation.

RESULTS AND DISCUSSION

Method development and optimized method
This method was accurate, specific, linear, precise, and suitable for the analysis of metoprolol and atorvastatin by RP-HPLC method. The HPLC instrument comprised a Waters HPLC with autosampler and UV detector. The Inertsil ODS-3 (4.6×150 mm, 5 µm) column is used. The ratio of mobile phase used is phosphate buffer 4.8 pH: acetonitrile (35:65v/v). Mode of separation is isocratic and its temperature of the column is ambient. The optimized chromatographic conditions are mentioned in Table 1 and chromatograms are shown in Figs. 4-7.

Assay
The assay study was performed for the metoprolol and atorvastatin in marketed tablet dosage form. For each determination, 3 times inject the solution into the column. The assay chromatogram is shown in Fig. 8 and the results are mentioned in Table 2.

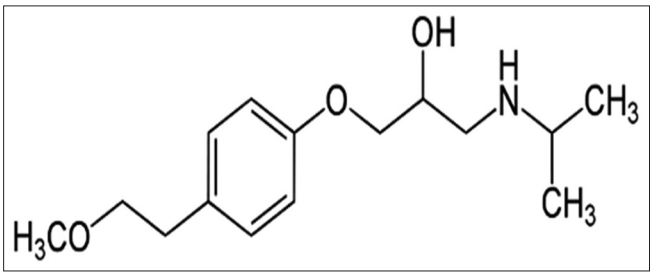

Fig. 1: Chemical structure of metoprolol

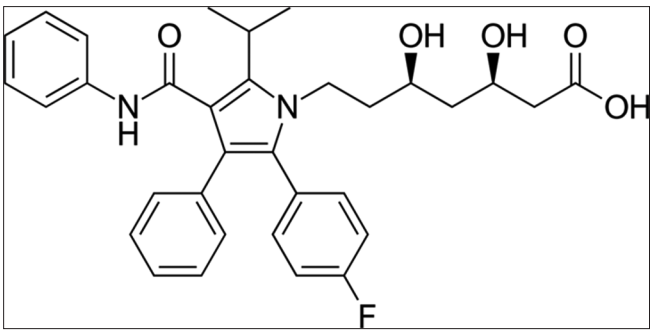

Fig. 2: Chemical structure of atorvastatin

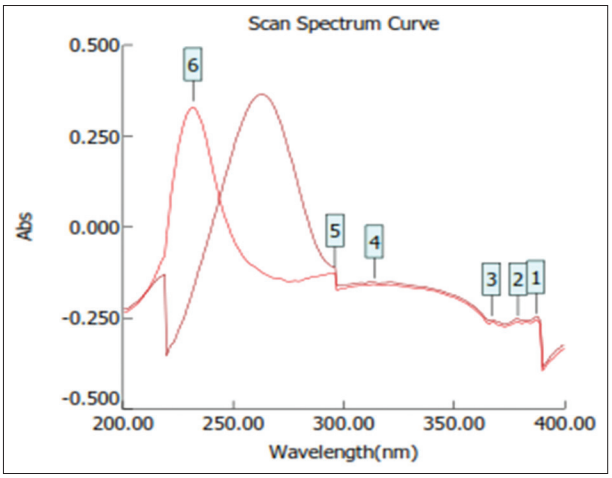

Fig. 3: Overlay spectrum for isosbestic point

Table 1: Optimized conditions

Optimization parameters	Method conditions
Stationary phase	Inertsil ODS-3 (4.6×150 mm, 5 µm)
Mobile phase	Phosphate buffer 4.8 pH and acetonitrile (35:65 v/v)
pH	4.8±0.02
Flow rate	1.0 ml/min
Analysis time each injection	15.0 min
Temperature of column	Ambient °C
Fixed injection loop volume	20 µl
Detection wavelength	244 nm
Drugs retention time	2.227 and 5.819 min

Method validation

This method was validated according to the ICH Q2 (R1) guidelines for various parameters.

Suitability

The mixed working solution was injected six replicates into the chromatographic column. The mean of suitability parameters was calculated from the obtained chromatogram. Results are tabulated in Tables 3 and 4.

Linearity and range

The linearity study was performed for the series concentrations 50–250 µg/ml and 10–50 µg/ml of metoprolol and atorvastatin,

Table 2: Assay data for marketed tablets

Tablet (Metpure St)	Label claim (mg)	Amount estimated* (mg)	Amount estimated (%)	Acceptance range (%)
Metoprolol	50	50.05	100.10	98–102
Atorvastatin	10	10.06	100.66	

*Mean of three determinations

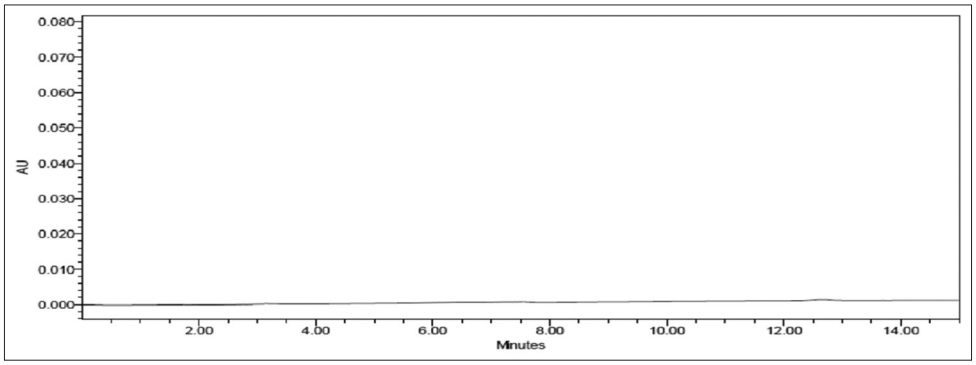

Fig. 4: Chromatogram for blank preparation

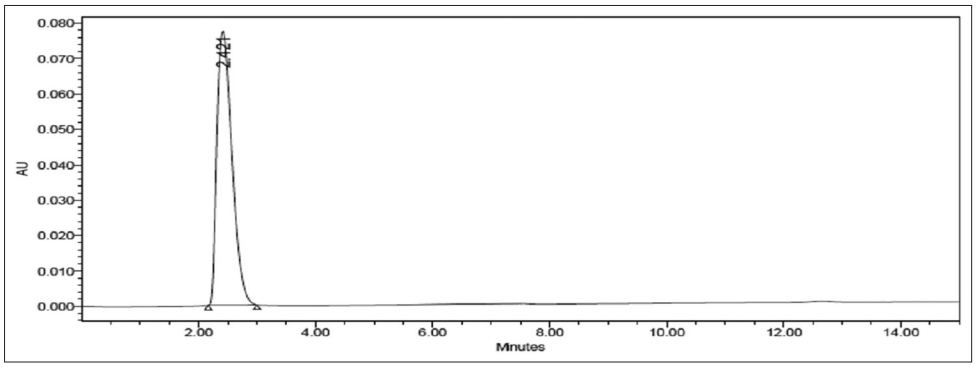

Fig. 5: Chromatogram for standard metoprolol

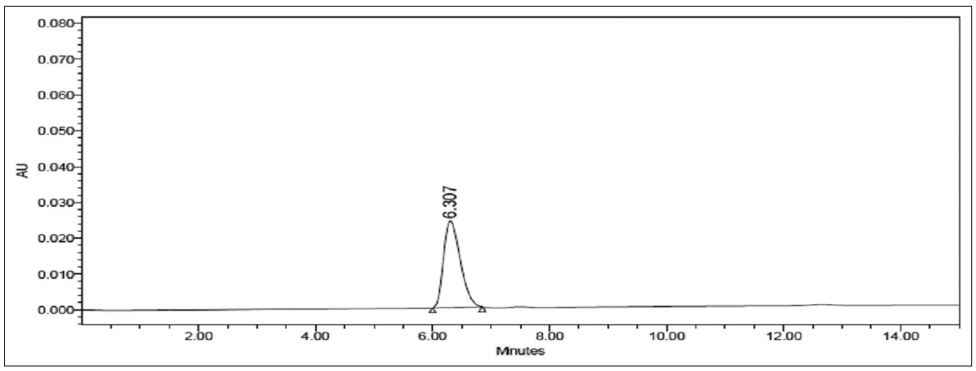

Fig. 6: Chromatogram for standard atorvastatin

respectively. The obtained values are tabulated in Tables 5 and 6. The graph for both the drugs is shown in Figs. 9-10 and overlay chromatogram in Fig. 11.

Accuracy

The accuracy study was performed for 80, 100, and 120% for metoprolol and atorvastatin. Each level was injected in triplicate into a chromatographic column. The area of each level was used for calculation of % recovery drug. The results are tabulated in Tables 7 and 8.

Table 3: System suitability for metoprolol and atorvastatin

Parameter	Metoprolol	Atorvastatin
Retention time (min)	2.227	5.819
Resolution (Rs. >2)	3.11	3.19
Asymmetry (T£2)	0.14	0.29
Theoretical plates	3941	2843
Tailing factor	1.54	1.84

Precision

The study of precision in this method was based on intraday and interday variations. The working standard solutions of metoprolol and atorvastatin have injected six replicas on the same day and on three different days for three different levels of concentrations. The mean and percentage relative standard deviation (% RSD) are tabulated in Tables 9 and 10. The results obtained all are within acceptable limits (% RSD <2).

LOD and LOQ

For metoprolol and atorvastatin, LOD was found to be 0.33 mg/ml and 0.21 mg/ml and LOQ was found to be 1.08 mg/ml and 0.69 mg/ml, respectively. The obtained values are tabulated in Table 11.

Robustness

It is a prediction of reliability for method development to maintain stable and unaffected the results are obtained by small changes

Table 4: System suitability (peak area and Rt) for metoprolol and atorvastatin

Injection	Peak area for metoprolol	Peak area for atorvastatin	Rt for metoprolol	Rt for atorvastatin
Injection-1	1,235,278	436,704	2.216	5.811
Injection-2	1,220,850	435,672	2.223	5.816
Injection-3	1,239,231	439,902	2.217	5.831
Injection-4	1,212,072	435,887	2.228	5.840
Injection-5	1,237,137	442,806	2.214	5.813
Injection-6	1,228,702	444,747	2.223	5.832
Average	1,228,878.3	439286.3	2.220	5.800
Standard deviation	10613.9	3843.8	0.00534478	0.00
% RSD	0.9	0.9	0.2	0.206454877

Rt: Retention time, RSD: Relative standard deviation

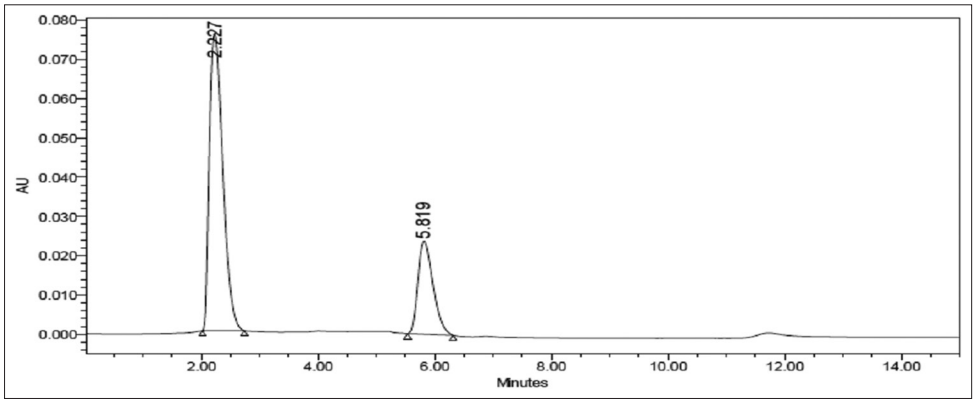

Fig. 7: Chromatogram for mixed standard metoprolol and atorvastatin at 244 nm from bulk drug

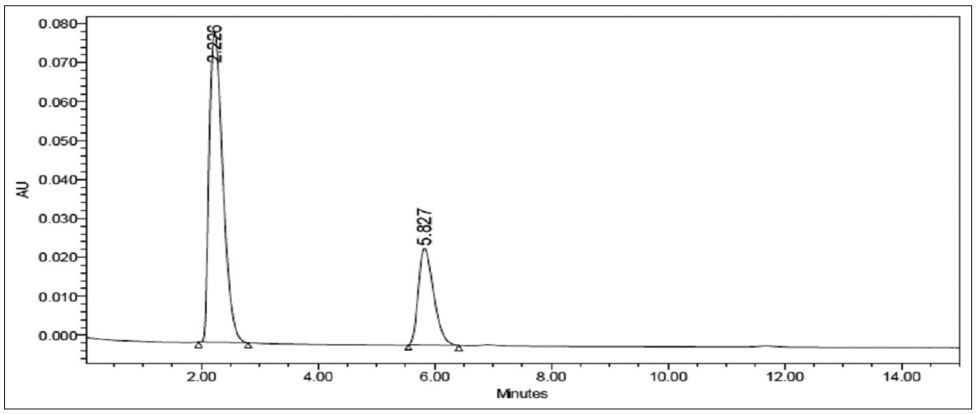

Fig. 8: Chromatogram for metoprolol and atorvastatin at 244 nm from pharmaceutical dosage form (Metpure St)

Table 5: Linearity results: (For metoprolol)

S. No	Linearity concentration (µg/ml)	Peak area
1	50	424,986
2	100	821,489
3	150	1,243,214
4	200	1,614,178
5	250	2,019,024
Correlation coefficient		0.9997

Table 6: Linearity results: (For atorvastatin)

S. No	Linearity concentration(µg/ml)	Peak area
1	10	144,310
2	20	297,966
3	30	437,053
4	40	572,746
5	50	724,791
Correlation coefficient		0.9995

Table 7: Accuracy data for metoprolol

Sample Id	Concentration (µg/ml)		% Recovery	Statistical data
	Pure drug	Amount added		
S_1: 80%	80	100	100.19	Mean=100.17%
S_2: 80%	80	100	100.17	SD=0.200083
S_3: 80%	80	100	100.16	% RSD=0.202152
S_4: 100%	100	100	100.39	Mean=100.40%
S_5: 100%	100	100	100.4	SD=0.33
S_6: 100%	100	100	100.43	% RSD=0.331525
S_7: 120%	120	100	100.67	Mean=100.68%
S_8: 120%	120	100	100.71	SD=0.33
S_9: 120%	120	100	100.68	% RSD=0.331159

RSD: Relative standard deviation

Table 8: The accuracy data for atorvastatin

Sample Id	Concentration (µg/ml)		% Recovery	Statistical data
	Pure drug	Amount added		
S_1: 80%	80	100	100.61	Mean=100.62%
S_2: 80%	80	100	100.64	SD=0.198578
S_3: 80%	80	100	100.62	% RSD=0.199884
S_4: 100%	100	100	100.84	Mean=100.84%
S_5: 100%	100	100	100.81	SD=0.032146
S_6: 100%	100	100	100.89	% RSD=0.03242
S_7: 120%	120	100	100.41	Mean=100.40%
S_8: 120%	120	100	100.46	SD=0.040415
S_9: 120%	120	100	100.45	% RSD=0.04068

Table 9: Intraday and interday precision for metoprolol standard solutions

Concentration (µg/ml)	Results			
	Intraday		Interday	
	Mean	% RSD	Mean	% RSD
80	80.04	0.24	79.76	0.22
100	100.45	0.35	100.12	0.33
120	119.16	0.15	120.20	0.18

RSD: Relative standard deviation

Table 10: Intraday and interday precision for atorvastatin standard solutions

Concentration (µg/ml)	Results			
	Intraday		Interday	
	Mean	% RSD	Mean	% RSD
80	80.05	1.02	79.59	0.98
100	99.94	0.74	100.09	0.56
120	119.97	0.35	120.006	0.32

RSD: Relative standard deviation

were made in method development. The robustness data conducted for variations in flow rate and percentage of composition in the mobile phase were performed. The obtained values are tabulated in Tables 12- 13.

Forced degradation studies

The data obtained in forced degradation studies reveal that the developed method is more stable in some stress conditions. Metoprolol was stable in thermal and photolytic (degradation) stress conditions, and the atorvastatin was comparatively stable in oxidation degradation. The obtained values are tabulated in Table 14, and chromatograms are shown in Figs. 12-16.

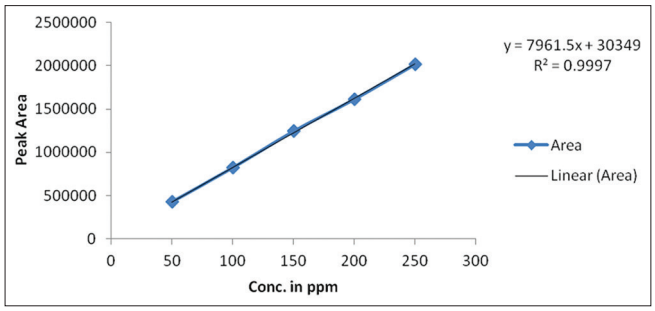

Fig. 9: Calibration curve of metoprolol

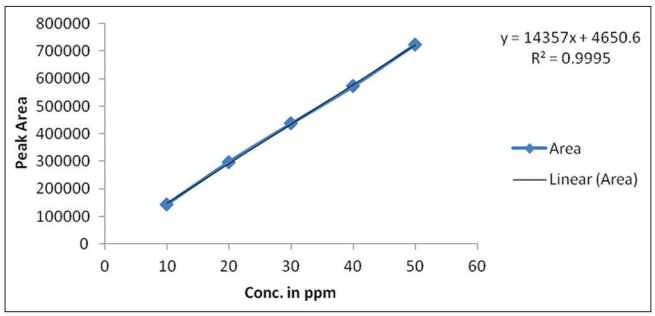

Fig. 10: Calibration curve of atorvastatin

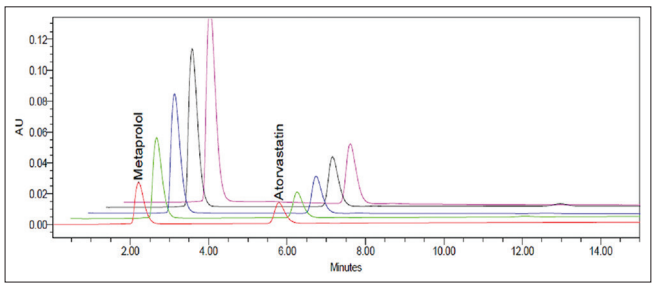

Fig. 11: Overlay report for linearity

CONCLUSION

Table 11: LOD and LOQ for metoprolol and atorvastatin

Parameter	Metoprolol	Atorvastatin
LOD	0.33	0.21
LOQ	1.08	0.69

LOD: Limit of detection, LOQ: Limits of quantification

Table 12: Robustness data for variation in flow rate

Drug	Flow rate (ml/min)	System suitability	
		Tailing factor	Theoretical plates
Metoprolol	0.9	1.53	3391.33
	*1	1.56	3399.02
	1.1	1.57	3418.14
Atorvastatin	0.9	1.82	2803.28
	*1	1.84	2843.08
	1.1	1.86	2892.46

*Results from assay standard

Table 13: Robustness data for variation in percentage of composition in the mobile phase

Drug	Percentage of composition in the mobile phase	System suitability	
		Tailing factor	Theoretical plates
Metoprolol	10% less	1.55	3445.74
	*Actual	1.56	3399.02
	10% more	1.52	3427.53
Atorvastatin	10% less	1.43	5082.74
	*Actual	1.36	5167.98
	10% more	1.43	5667.09

*Results from assay standard

Table 14: Degradation results for metoprolol and atorvastatin

Sample name	Metoprolol		Atorvastatin	
	Area	% Degraded	Area	% Degraded
Standard	1214803		426473	
Acid	1196736	1.49	416562	2.32
Base	1175633	3.22	410776	3.68
Peroxide	1097863	9.63	407623	4.42
Thermal	1167563	3.89	403572	5.37
Photo	1165552	4.05	398772	6.50

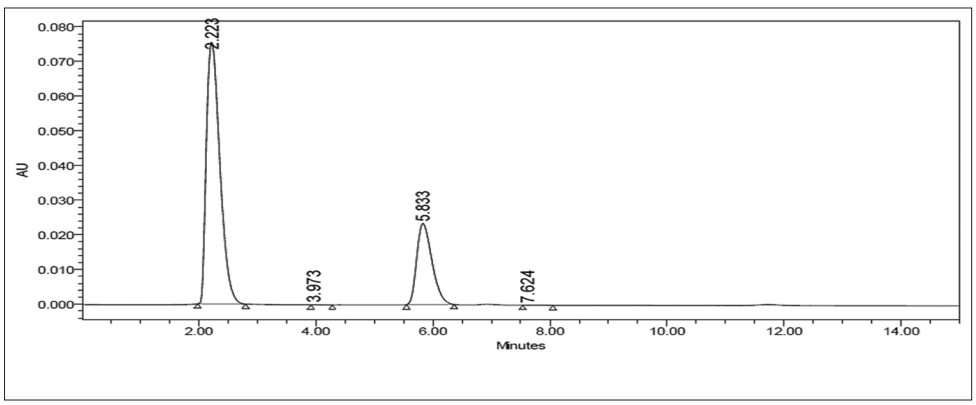

Fig. 12: Acidic degradation

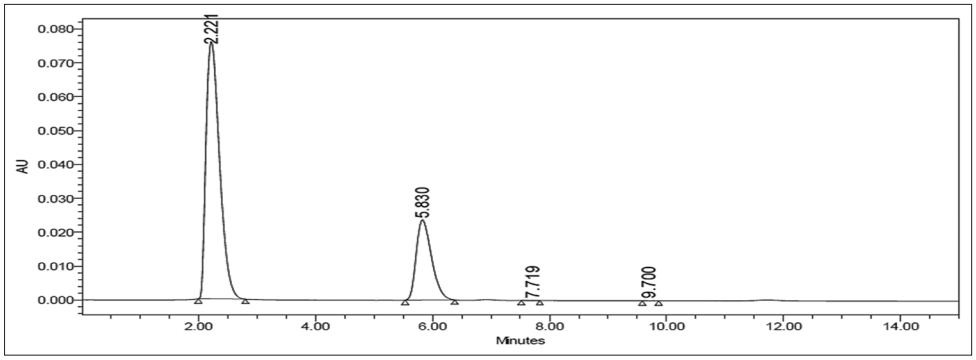

Fig. 13: Alkaline degradation

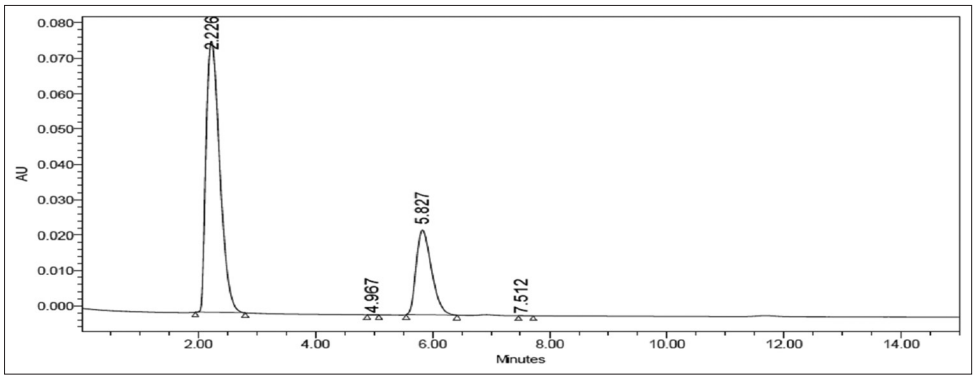

Fig. 14: Thermal degradation

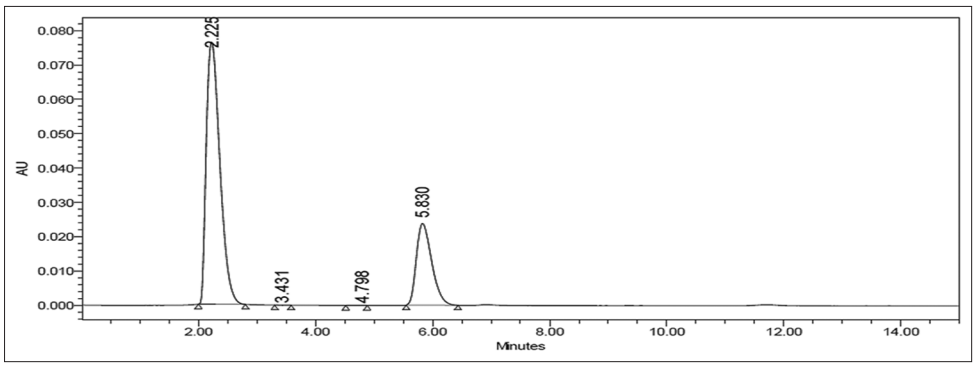

Fig. 15: Oxidative degradation

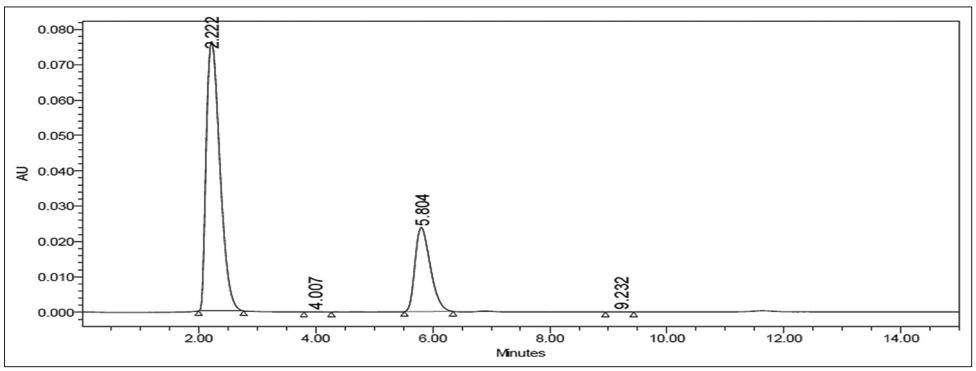

Fig. 16: Photolytic degradation

The obtained results for this method validation are within acceptance criteria. This method was more economical and stable. This method could selectively quantify metoprolol and atorvastatin in a pharmaceutical tablet dosage form. From the obtained experimental data, the developed method is more accurate, precise, and selective, so this method was suitable for routine analysis successfully for this

combination in its bulk and marketed formulations by RP-HPLC using biorelevant dissolution media (FaSSIF).

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