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HPLC DETERMINATION OF CARVEDILOL AND ATORVASTATIN CALCIUM IN THEIR BULK AND DOSAGE FORMS

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ABSTRACT

An isocratic HPLC method had been developed for rapid simultaneous separation and determination of carvedilol and atorvastatin calcium in pure form and pharmaceutical preparation. Separation was carried out on a Hypersil gold C18 (15um, 100x4.6mm) column. Mobile phase composed of methanol, acetonitrile and 0.1% ortho phosphoric acid in the ratio of 40:45:15(v/v/v) with 0.6 ml per min flow rate and detection was at 254 nm. Linearity was obtained in the concentration range of 1-90 (µg. mL^{-1}) for both drugs. The method was applied for the determination of drugs in both bulk and pharmaceutical formulation and was validated when obtained results were compared with reference methods.

KEYWORDS

Carvedilol, Atorvastatin calcium, Hypersil gold, Mobile phase and RP- HPLC method.

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INTRODUCTION

Carvedilol is chemically, (2RS)-1-(9H-Carbazol-4-yloxy)-3- [[2-(2-methoxy phenoxy) ethyl] amino] propan-2-ol (Figure No.1). It has a molecular formula of $C_{24}H_{26}N_2O_4$ and a molecular weight of 406.5g/mol[1]. Carvedilol belongs to a group of medicines called beta-adrenergic blocking agents that are indicated for the treatment of hypertension, angina pectoris, and heart failure[2]. It has vasodilating properties, which are attributed mainly to its blocking activity at $alpha_1$ receptors at higher doses calcium-channel blocking activity may contribute. It also has antioxidant properties[3]. Several analytical methods, including

spectrophotometric methods[4-7],spectrofluorometric[8], chromatographic method[9-12] and electro chemical methods[13,14] have already been reported for its determination, either alone or in combination with other drugs.

Atorvastatin Calcium is chemically, [(3R,5R)-7- [3-(phenyl carbamoyl)-5-(4-fluorophenyl)-2-isopropyl 4-phenyl-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt] (figureNo.1). It has a molecular formula of $C_{66}H_{68}CaF_2N_4O_{10}$ and a molecular weight of 1155.34 g/mol[15]. It acts by inhibiting the enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-co A) reductase[16].

Several analytical methods, including spectrophotometric methods[17-21], spectrofluoro-metric[22], chromatographic methods[23-30] and potentiometric method[31] have already been reported for its determination, either alone or in combination with other drugs.

Hypersil gold columns achieve exceptional peak shape and resolution for HPLC and LC/MS applications. These endcapped, ultrapure, silica-based columns deliver significant reduction in peak tailing, excellent resolution, efficiency, sensitivity and confidence in the accuracy and quality of analytical data[32].

Some HPLC methods using Hypersil gold columns has been reported, like determination of some cephalosporins[32], determination of Miconazole and its impurities[33], Determination of Cotinine in Urine[34] and determination of azithromycin in human plasma[35].

In this part, an isocratic HPLC method had been developed for rapid simultaneous separation and determination of carvedilol and atorvastatin calcium in pure form within 6 minutes. Separation was carried out on a Hypersil gold (15um, 100x4.6mm) using a mobile phase of methanol: acetonitrile:0.1% ortho-phosphoric acid at pH 1.8 (40:45:15 v/v/v) at ambient temperature. The flow rate was 0.6 ml/min and maximum absorption was measured at 254nm.

MATERIAL AND METHOD

Apparatus

HPLC apparatus equipped with a Surveyor quaternary pump with Intel vacuum degasser (Thermo Scientific Co. USA), Surveyor auto sampler plus (Thermo Scientific Co., USA), Surveyor photodiode array detector (PAD) (Thermo Scientific Co. USA). Computer with a software chromo quest 5 (Surveyor Thermo Scientific Co. USA), for data collection and analysis, Hypersil gold C18 (15um, 100x4.6mm) column (Thermo Scientific Co. USA). Auto sampler vials 1.8 ml screw cap, Thermo Scientific, USA.

Consort P400® digital pH-meter for pH adjustment.

Material and Reagents

All solvents and reagents were of an HPLC analytical grade (methanol, acetonitrile and orthophosphoric acid were supported from Romil, England).

Carvedilol was provided by Global Napi Pharmaceuticals, Egypt, purity 99%. Carvipress ® tablet, labeled to contain 25mg carvedilol per tablet, batch No.A12202 (Global Napi Pharmaceuticals, Egypt).

Atorvastatin Calcium was provided by EIPICO Company, Egypt, purity 99.06%. Ator ® tablet labeled to contain 40 mg atorvastatin calcium per tablet, batch No.1404653 (EIPICO, Egypt).

Mobile phase was a freshly prepared ternary mixture of methanol: acetonitrile: 0.1% ortho phosphoricacid at pH 1.8 (40:45:15 v/v/v), filtered and degassed using 0.45µm membrane filter.

GENERAL PROCEDURES

Preparation of standard drug solutions

Standard solutions 100 $\mu g.ml^{-1}$ was prepared individually by dissolving 10 mg of each pure drug in 100 ml of the mobile phase.

Procedure for authentic powder

10 mg of each drug were dissolved in 100 ml of the mobile phase, filtered into 100 - ml measuring flask and completed to volume with the mobile phase. Appropriate mixed dilution of the standard stock solutions was done in 10 - ml volumetric flask to get final concentration of 60 $\mu g.ml^{-1}$ for all drugs. A

10 µl of the mixture was injected into the column and the chromatogram was obtained at 254nm.

Procedure for marketed products

Ten tablet of each formulation were powdered and weighed. An accurately amounts of the powder equivalent to 10 mg of each drug were dissolved in 100 ml of the mobile phase, filtered into 100 - ml measuring flask and completed to volume with the mobile phase. The procedure was then completed as previously mentioned under the procedure for authentic powder.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

All chromatographic conditions are illustrated in (TableNo.1). The chromatographic detection was performed at 254nm using Surveyor photodiode array detector (PAD) (Thermo Scientific Co. USA). The method was performed on a Hypersil gold® C18 (15um, 100x4.6mm) column (Thermo Scientific Co. USA). It was observed that when a combination of the two drugs was injected, carvedilol and atorvastatin calcium together gave a mixed peak. Chromatographic conditions were optimized by changing the mobile phase composition. Different experiments were performed to optimize the mobile phase but adequate separation of drugs could not be achieved. By altering mobile phase composition, flow rate and wave length detection as seen in (Figures No. 2-4), a good separation was achieved. The optimized mobile phase was determined as a mixture of methanol: acetonitrile: 0.1% ortho phosphoric acid (40:45:15 v/v/v) at a flow rate of 0.6ml/min. Under these conditions, carvedilol, and atorvastatin calcium were eluted at 2.875 and 4.380 minutes respectively with a run time of 6 minutes.

A typical chromatogram for simultaneous estimation of the two drugs obtained by using the aforementioned mobile phase is illustrated in (Figures No.5,6) for authentic mixture and pharmaceutical formulations respectively.

Method Validation

The developed methods were validated according to international conference on harmonization guidelines ICH[36].

Linearity

Eleven different concentrations of a mixture of the two drugs were prepared for linearity studies. The response was measured as peak area. The calibration curves obtained by plotting peak area against concentration showed linearity in the concentration range of 1 -90 $\mu g.ml^{-1}$in case of carvedilol and atorvastatin calcium. Linear regression equations of carvedilol and atorvastatin calcium were found to be y = 95833x + 91498 and y = 14394x + 16758, respectively and the regression coefficient values (r) were found to be 0.9998, and 0.9999, respectively indicating a high degree of linearity for all drugs.

Accuracy

The accuracy of the proposed method was determined by investigating the recovery of drugs at concentration levels covering the specified range (three replicates of each concentration). The results showed excellent recoveries (Table No.2).

Precision

Intraday precision was evaluated by calculating standard deviation (SD) of five replicate determinations using the same solution containing pure drug. The SD values revealed the high precision of the methods. For inter-day reproducibility on a day - to - day basis, a series was run, in which the standard drug solutions were analyzed each for five days. Results showing in (Table No.5).

Specificity

The specificity studies revealed the absence of any excipient or impurity interference, since none of the peaks appeared at the same retention time of carvedilol and atorvastatin calcium as shown in (Figure No.5).

Limit of Detection and Limit of Quantification

For determining the limit of detection (LOD.) and limit of quantification (LOQ.), the method based on signal to noise ratio (3:1 for LOD. and 10:1 for

LOQ.) was adopted. The limit of detection was 0. 282, 0.272 µg.ml-1 for carvedilol and atorvastatin calcium respectively, while the limit of quantitation was 0.94, 0.92 µg.ml-[1]for carvedilol and atorvastatin calcium respectively (Table No.2).

Robustness

The robustness of the method was evaluated by making small changes in one parameter keeping the other chromatographic conditions constant such as the flow rate and mobile phase ratio where the effect of the changes was studied on the percent recovery of drugs. The changes had a negligible influence on the results, data are summarized in (Table No.6).

Applications

Some Pharmaceutical formulations containing stated drugs have been successfully analyzed by the proposed method. Results obtained were compared to those obtained by applying reference methods [10,30] where Student's t-test and F-test were performed for comparison (Table No.4). The calculated t and F values were less than tabulated values for the four drugs which in turn indicate that there is no significant difference between proposed method and reference ones relative to precision and accuracy.

Table No.1: Chromatographic conditions for the proposed HPLC method

S.No	Parameters	Conditions
1	Column	Hypersil gold® C18 (15um, 100x4.6mm) column
2	Mobile phase	Isocratic ternary mobile phase methanol: acetonitrile: 0.1% ortho phosphoric acid in the ratio of 40: 45:15v/v/v, filtered and degassed using 0.45µm membrane filter
3	UV detection, nm	254
4	Flow rate, ml/min	0.6
5	Injected volume, µl	10
6	Pressure, MPa	11
7	Temperature	Ambient (25±5 ℃)
8	Retention time Carvedilol Atorvastatin calcium	 2.875 4.380

Table No.2: Results and characteristic parameters for the simultaneous determination of Carvedilol and Atorvastatin calcium by the proposed HPLC method

S.No	Parameters	CARVEDILOL			ATORVASTATIN CALCIUM		
		Taken µg/ml	Found µg/ml	Recovery %	Taken µg/ml	Found µg/ml	Recovery %
1		1	1.0071165	100.71165	1	0.9870085	98.700847
2		5	4.9267371	98.534742	5	4.9145477	98.290954
3		10	9.8755543	98.755543	10	9.9934695	99.934695
4		20	19.872653	99.363267	20	20.124218	100.62109
5		30	29.998038	99.993461	30	30.447547	101.49182
6		40	40.515616	101.28904	40	40.097749	100.24437
7		50	49.720096	99.440193	50	49.646729	99.293456
8		60	60.718364	101.19727	60	59.41427	99.023783
9		70	69.211378	98.873397	70	70.22989	100.32841
10		80	80.418707	100.52338	80	79.58865	99.485810
11		90	89.734590	99.705100	90	90.56607	100.62896
12	Mean recovery*			99.853369			99.822201
13	S D			0.9700344			0.956882
14	RSD			0.9714589			0.958586
15	SE			0.2921790			0.2882174
16	Variance			0.9409668			0.9156225
17	Slope			95832.757			14394.325
18	Intercept			91498			16758
19	LOD.			0.2824178			0.2719151
20	LOQ.			0.9413926			0.9063836
20	Apparent Molar absorbtivity L. Mol $^{-1}$. Cm $^{-1}$			439130628			192104546

* Average of three independent procedures.

Table No.3: Application for the determination of Carvedilol (Carvipress 25mg tablets) and Atorvastatin calcium (Ator 40 mg tablets) in pharmaceutical dosage forms

Items	CARVEDILOL			ATORVASTATIN CALCIUM		
	Conc. taken µg/ml	Conc. found µg/ml	%Recovery	Conc. taken µg/ml	Conc. found µg/ml	Recovery*%
	10	9.8430	98.43	10	9.9919	99.92
	20	19.9648	99.82	20	20.0152	100.07
	30	30.1002	100.33	30	29.8074	99.36
	40	40.5336	101.33	40	40.378	100.94
	50	49.5587	99.12	50	49.812	99.62
Mean*			99.808			99.98
N			5			5
SD			1.116			0.6036
RSD			1.118			0.6037
V			1.245			0.364
SE			0.498			0.2695

*Mean of three different experiments.

Table No.4: Statistical analysis of results obtained by the proposed HPLC method applied on Carvipress® 25mg tablets and Ator® 40 mg tablets compared with the reference methods

S.No	Drug	Carvedilol		Atorvastatin calcium	
1	Statistics	Reference method [10]	Proposed method	Reference method [30]	Proposed method
2	N	5	5	5	5
3	Mean recovery*	100.31	99.808	100.25	99.98
4	SD	0.69	1.116	0.9524	0.6036
5	variance	0.4761	1.245	0.907	0.364
6	T –test		0.855 (2.306) a		0.536 (2.306) a
7	F- ratio		2.615(6.338)b		2.492 (6.338) b

*Average of three experiments.

a and b are the Theoretical Student t-values and F-ratios at p=0.05.

Table No.5: Results of the intraday and inter-day precision for the determination of Carvedilol and Atorvastatin calcium by proposed HPLC method

S.No	Drug	conc. µg/ ml	Intra-day		Inter-day	
			mean± SD	RSD	mean± SD	RSD
1	Carvedilol	10	99.74 ± 0.83	0.84	99.58± 0.94	0.94
		30	99.62± 0.703	0.705	99.85± 0.62	0.62
		60	100.95 ± 0.34	0.33	101.11± 0.22	0.22
2	Atorvastatin	10	99.86 ± 0.902	0.904	99.65 ± 0.84	0.84
		30	101.78 ± 0.97	0.95	101.20 ± 0.75	0.74
		60	98.71 ± 0.16	0.16	98.66 ± 0.34	0.34

Table No.6: Results of the robustness for the determination of Carvedilol and Atorvastatin calcium by HPLC method

S.No	Parameters	% of recovery ± SD	
		Carvedilol	Atorvastatin calcium
1	Flow rate 0.59	101.18 ±0.97	98.85 ±0.96
2	Flow rate 0.61	101.37 ±0.99	98.66 ±0.97
3	Mobile phase ratio 39:46:15	100.91 ±0.94	98.93 ±0.96
4	Mobile phase ratio 41:44:15	101.35 ±0.99	98.71 ±0.96

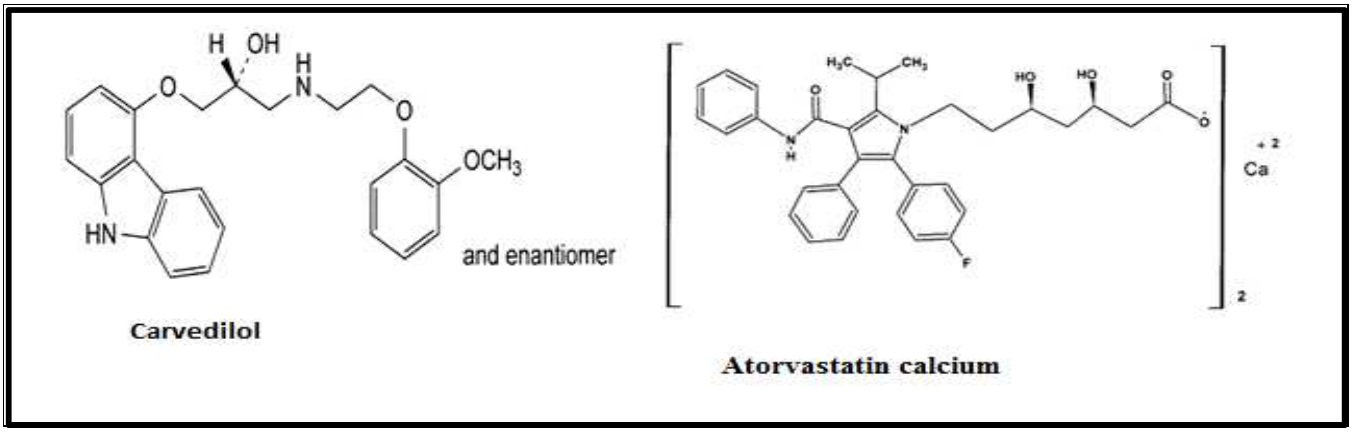

Figure No.1: Chemical structures of Carvedilol and Atorvastatin calcium

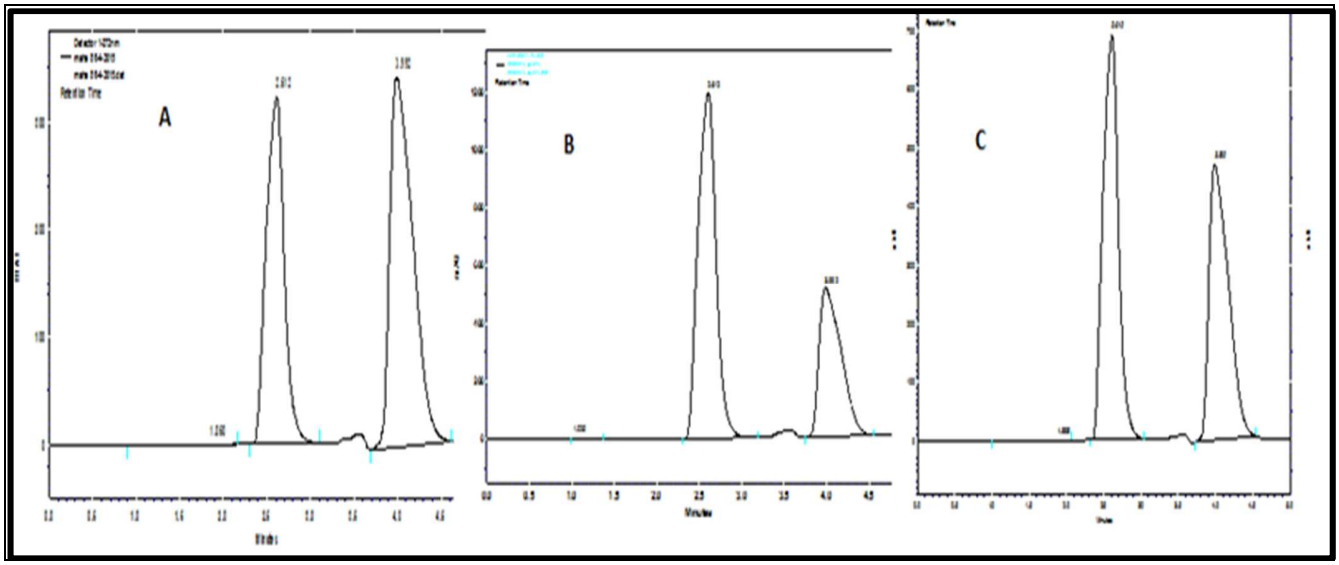

Figure No.2: HPLC Chromatogram of Authentic mixture of 60 µg.ml^{-1} (Carvedilol and Atorvastatin calcium) respectively at different UV detection

Column: Hypersil gold C18 (15um, 100x4.6mm) column.
Mobile phase: methanol: acetonitrile: 0.1 % ortho phosphoric acid at pH 1.8 (40:45:15 v/v/v).
Flow rate: 0.6 ml/min.
pH: 1.8.
UV detection: **A** (272 nm), **B** (215 nm) and **C** (254 nm).

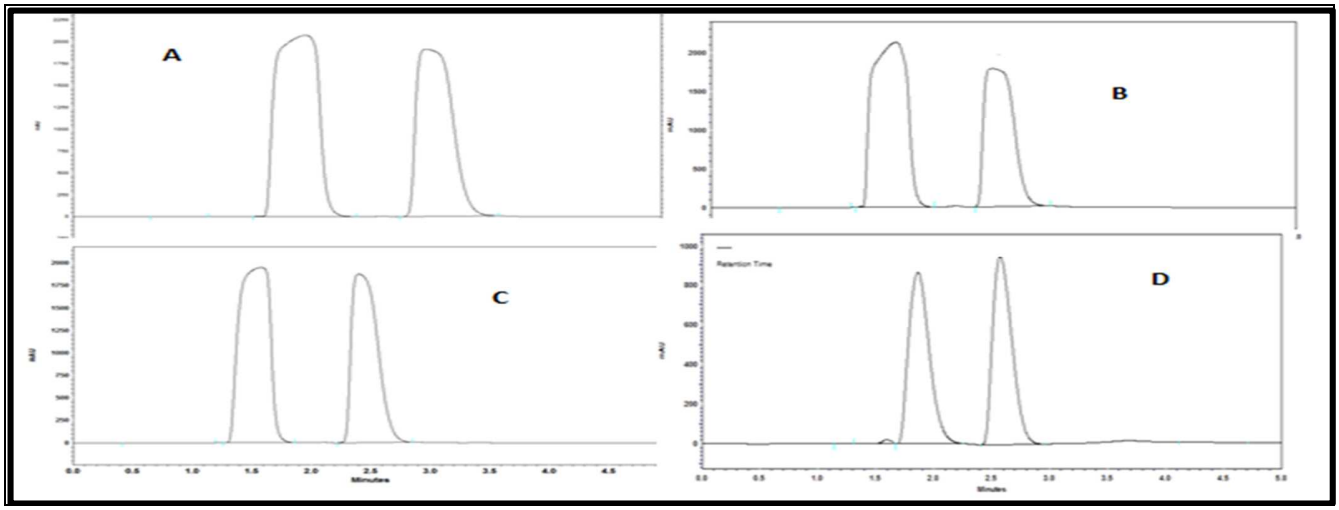

Figure No.3: HPLC Chromatogram of Authentic mixture of 60 µg.ml^{-1} (Carvedilol and Atorvastatin calcium) respectively at different percentages of mobile phase

Column: Hypersil gold C18 (15um, 100x4.6mm) column.
Mobile phase: methanol: acetonitrile: 0.1 % ortho phosphoric acid at pH 1.8.
A (10:75:15), **B** (20:65:15), **C** (30:55:15) and **D** (40:45:15).
Flow rate: 0.6 ml/min.
pH: 1.8.
UV detection: 254 nm.

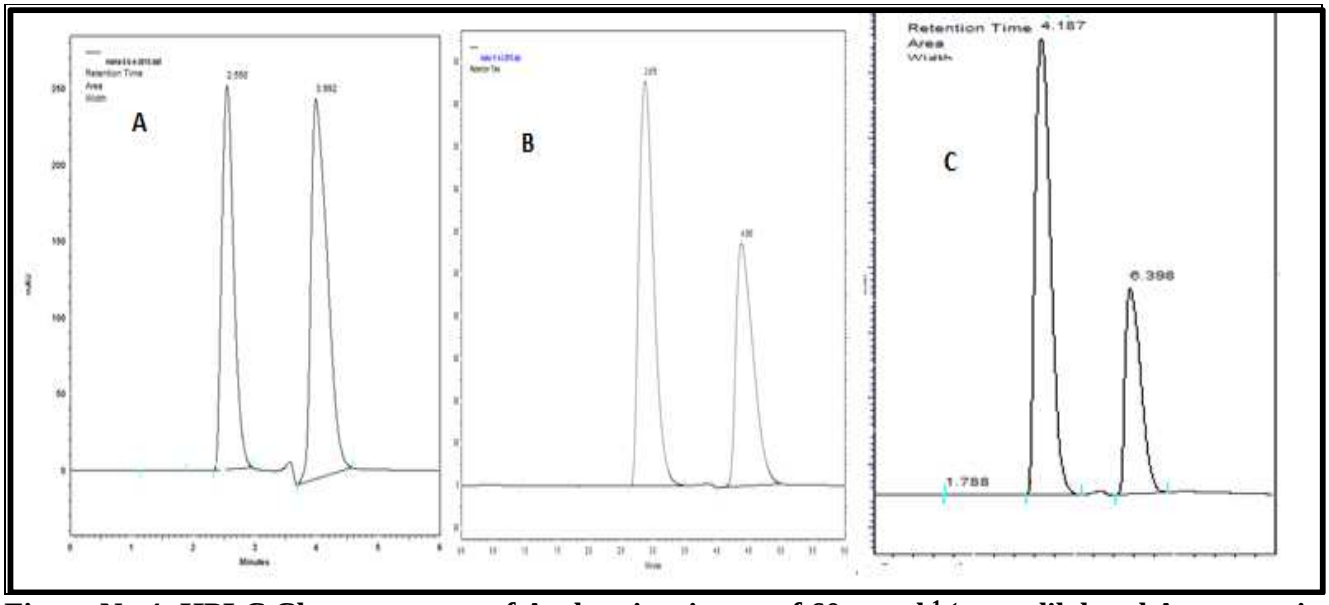

Figure No.4: HPLC Chromatogram of Authentic mixture of 60 µg.ml^{-1} (carvedilol and Atorvastatin calcium) respectively at different flow rates

Column: Hypersil gold C18 (15um, 100x4.6mm) column.
Mobile phase: methanol: acetonitrile: 0.1 %ortho phosphoric acid (40: 45:15 v/v/v/)
Flow rate: **A** (0.8 ml/min), **B** (0.6 ml/min) and **C** (0.4 ml/min).
pH: 1.8.
UV detection: 254 nm.

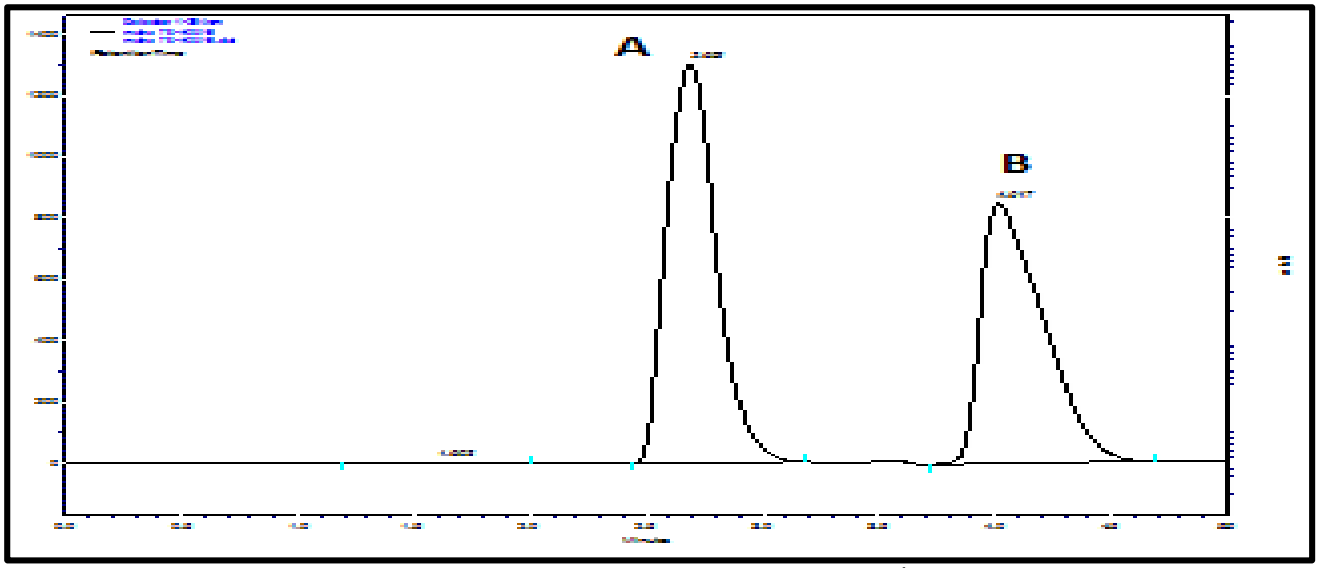

Figure No.5: HPLC Chromatogram of authentic mixture of 60 µg.ml^{-1} Carvedilol (A) and Atorvastatin calcium (B)

Column: Hypersil gold C18 (15um, 100x4.6mm) column.
Mobile phase: methanol: acetonitrile: 0.1 % ortho phosphoric acid (40:45:15 v/v/v).
Flow rate: 0.6 ml/min.
pH: 1.8.
UV detection: 254 nm.

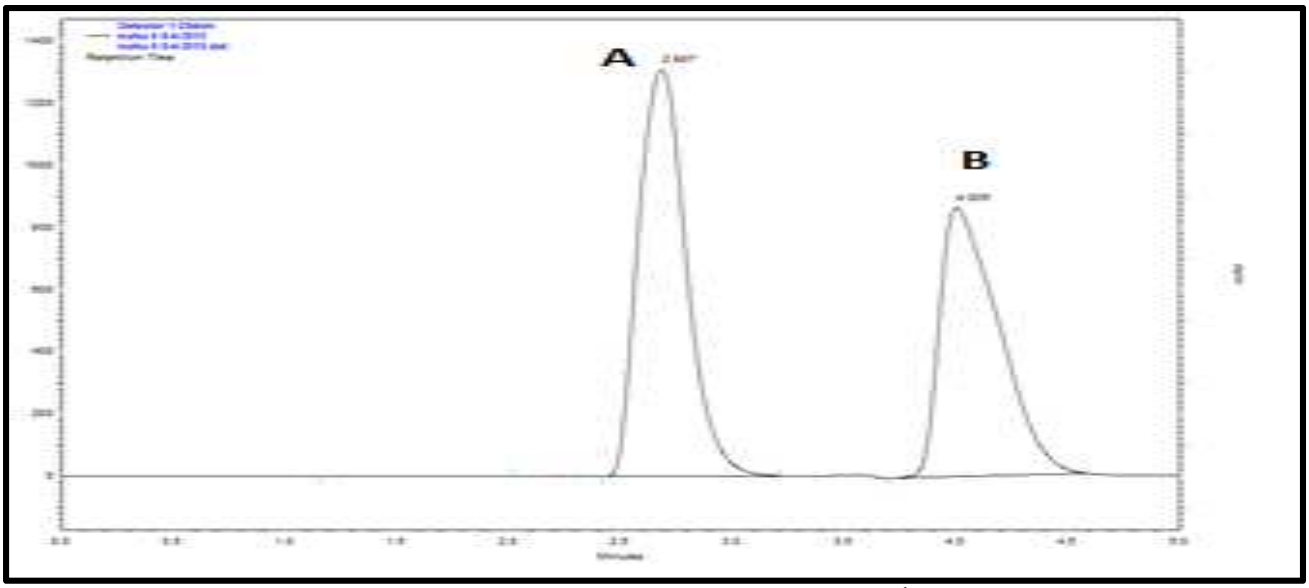

Figure No.6: HPLC Chromatogram of marketed mixture of 60 µg.ml^{-1} carvedilol (A) and Atorvastatin calcium (B)

Column: Hypersil gold C18 (15um, 100x4.6mm) column.
Mobile phase: methanol: acetonitrile: 0.1 % ortho phosphoric acid (40:45:15, v/v/v).
Flow rate: 0.6 ml/min.
pH: 1.8.
UV detection: 254 nm.

CONCLUSION

An RP-HPLC method for rapid simultaneous estimation of carvedilol and atorvastatin calcium within 6 minutes was developed and validated. The amounts obtained by the proposed method are between 98.3% and 101.6%, within the acceptance level of 95% to 105%. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. Linearity was observed over a concentration range of 1 to 90ug.ml-1 for the two drugs. The method has been successfully applied for the analysis of marketed formulation. It can be used for the routine analysis of formulations containing any one of the above drugs or their combinations without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations in addition to reduced analysis time due to column.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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