Original Article

Method Development And Validation Of Stability Indicating Rp-Hplc Method For The Estimation Of Atrovastatin And Fenofibrate Combination In Tablet Dosage Form

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Abstract

A stability indicating RP-HPLC method was developed and validated for the determination of Atorvastatin and Fenofibrate in tablet dosage form. The method was carried out using Agilent C18 column (100 mm×4.6 mm, 2.5μ) with mobile phase consisting 0.1 % ortho-phosphoric acid and Methanol (25:75 v/v), at a flow rate of 1.0 mL/min and the effluent monitored at 257 nm. The retention time of Atorvastatin and Fenofibrate were found to be 5.254 and 7.341 min respectively. Linearity was observed over the concentration range of 1-5 μg/mL for Atorvastatin and 16-80 μg/mL for Fenofibrate. The percentage recoveries of Atorvastatin and Fenofibrate in the marketed dosage form found to be 100.27 and 99.55 respectively. The reliability and analytical performance of the proposed method were statistically validated for linearity, precision, accuracy, and ruggedness, Limit of detection, and quantification limits. The drug was subjected to stress conditions including acidic, alkaline, oxidation, photolysis and humidity degradation. The drug is more sensitive towards oxidation degradation. The method was validated as per ICH guidelines.

Keywords: Atorvastatin, Fenofibrate, RP-HPLC, Stressed degradation, Validation.

INTRODUCTION:

Atorvastatin calcium is (β R, δ R)-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methyl ethyl)-3-phenyl-4-((phenyl amino) carbonyl)-1H-pyrrole-1-hepatonoic acid, a HMG CoA reductase inhibitors and fenofibrate is 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester.[1] It is indicated for the treatment of hypercholesterolemia and mixed dyslipidaemia. The survey of literature revealed some HPLC methods for determination of Atorvastatin and Fenofibrate in tablet separately and a simultaneous method for estimation of Atorvastatin and aspirin in their combined dosage form by HPLC. A high performance liquid chromatographic method for determination of fenofibrate in tablets is also reported. [2] However, no HPLC method for the simultaneous estimation of Atorvastatin and Fenofibrate in combined dosage forms has been reported so far. The present work describes the development of simple, precise and accurate isocratic reverse phase HPLC method for simultaneous estimation of Atorvastatin and Fenofibrate in tablets. [3]

CHEMICALS AND REAGENTS:

Methanol, water, Acetonitrile is of HPLC grade and ortho-phosphoric acid (Analytical grade) , Water and 0.45 μm nylon membrane filters .

INSTRUMENTATION:

The separation was carried out on HPLC system with Agilent 1100 Diode array detector (DAD). Agilent C18 column (100 x 4.6 mm, 2.5 μ).

CHROMATOGRAPHIC CONDITIONS:

Chromatographic separation was carried out on Agilent C18 column (100 x 4.6 mm, 2.5 μ) using mobile phase 0.1% ortho-phosphoric acid and Methanol (25:75 v/v) was operated on isocratic mode. In this method, 1.0 mL/min flow rate was used for the separation and detection was performed at 257 nm. The injection volume was 20 μL.

PREPARATION OF STOCK SOLUTIONS:

The standard stock solutions were prepared separately by transferring 5 mg of Atorvastatin, 80 mg of Fenofibrate into 100 mL of standard volumetric flask. To that about 50 mL of diluent was added, the solution. Further dilutions were made with diluent to get the final concentrations of Atorvastatin (1-5μg/mL) and Fenofibrate (16-80 μg/mL).

PREPARATION OF SAMPLE SOLUTION:

Twenty tablets each Atorvastatin and Fenofibrate were weighed. And add into 100 mL of standard volumetric flask containing 50 mL of diluent, sonicate for 20 min and made up to the volume. Further secondary dilutions were made with diluent to final concentrations of Atorvastatin 50 μg/mL and Fenofibrate 800 μg/mL. Quantification was achieved by peak area ratio method with reference to the standards.

METHOD VALIDATION:

The developed HPLC method was validated according to International Conference on Harmonization (ICH) guidelines. The method was validated for the parameters like linearity, accuracy, and precision, limit of detection (LOD) and limit of quantification (LOQ).[5]

SYSTEM SUITABILITY:

System suitability test is used to verify that the resolution and reproducibility were adequate for the analysis performed. Parameters calculated for system suitability were % RSD (relative standard deviation) of retention time, area, number of theoretical plates and resolution.

LINEARITY:

The purpose of the test for linearity is to demonstrate that the entire analytical system (including detector and data acquisition) exhibits a linear response and is directly proportional over the relevant concentration range for the target concentration of the analyte. A series of combination of standard dilutions were prepared over a concentration range of 1-5 μg/mL and 16-80 μg/mL of Atorvastatin and Fenofibrate respectively. The linearity was evaluated by linear regression analysis, which was calculated by least square method.

PRECISION AND ACCURACY:

Precision is the measure of closeness of true value. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Accuracy is the degree of agreement between a measured value and the accepted reference value. The accuracy was determined by recovery studies of Atorvastatin and Fenofibrate, known amount of standard was added to the pre-analysed sample and subjected to the proposed HPLC analysis.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION:

The sensitivity of measurement of Atorvastatin and Fenofibrate by use of the proposed method was estimated in terms of the Limit of quantification (LOQ) and Limit of Detection (LOD). Limit of detection (LOD) and quantification (LOQ) were estimated from both linearity calibration curve method and signal to noise ratio method. These two parameters were calculated using the formula based on the standard deviation of the response and the slope.

ROBUSTNESS:

The robustness of the method was studied by small but deliberate variations from the optimized conditions in wavelength (± 1 nm) and flow rate (± 0.1 mL/min). One factor at a time was changed at a concentration level of 5 μg/mL of Atorvastatin and 80 μg/mL of Fenofibrate, the peak area obtained with each solution was measured and % RSD was calculated.

SYSTEM SUITABILITY:

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Atorvastatin and Fenofibrate system suitability parameters were studied. The result shown in below

FORCED DEGRADATION STUDIES:

The specificity of the method was demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, reductive, thermal and photolytic degradation. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient.

ACID DEGRADATION:

The Atorvastatin and Fenofibrate mixture was treated with 5 mL of 0.1N HCl and heated on water bath and kept aside at 25 °C for 1 hr. The mixture was cooled to room temperature and volume was made up to 10 ml with diluent. [7]

BASE DEGRADATION:

The Atorvastatin and Fenofibrate mixture was treated with 5 mL of 0.1N NaOH and heated on water bath and kept aside at 25 °C for 1 hr. The mixture was cooled to room temperature and solution was filtered using 0.45 µ syringe filter and sonicated for 5 min and diluted to volume with 10 mL of diluent and mixed.

OXIDATION DEGRADATION:

The Atorvastatin and Fenofibrate mixture was treated with 5 mL of 3% H2O2 and heated on boiling water bath to remove the excess of hydrogen peroxide. The mixture was cooled to room temperature and diluted to volume with diluent and mixed.

PHOTOLYTIC DEGRADATION:

Sample mixture of Atorvastatin and Fenofibrate was exposed to sunlight for 24 hr. Sample was withdrawn, dissolved in mobile phase and volume was made up to 10 mL with diluent. [7]

HUMIDITY DEGRADATION:

The Atorvastatin and Fenofibrate mixture was treated at 75% relative humidity for 24 hr. The volume was made up to 10 mL with diluent.

RESULTS AND DISCUSSION

The aim of the present investigation is to develop a sensitive, precise and accurate high performance liquid chromatographic method for the determination of Atorvastatin and Fenofibrate in pharmaceutical formulations. Agilent C18 column (100 x 4.6 mm, 2.5 µ) was found to be optimum. A mixture of 0.1% ortho-phosphoric acid in Water and Methanol in the ratio of 75:25 v/v was selected as optimum mobile phase at flow rate of 1.0 mL/min. The proposed RP-HPLC method was successfully used for the simultaneous determination of the Atorvastatin and Fenofibrate in the formulation by UV detection at 257 nm. System suitability test was applied to representative chromatograms for various parameters such as tailing factor and number of theoretical plates was determined. The results reported (**Table 1 and Fig. 2**) [1][7]

Table No 1: Linearity data for Atorvastatin

Method	Concµg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	10	1108.91	1127.11	1118.01	12.87	1.15
	20	2272.01	2278.06	2275.03	4.28	0.19
	30	3350.29	3350.01	3350.15	0.20	0.01
	40	4402.88	4388.26	4395.57	10.34	0.24
	50	5427.35	5435.1	5431.22	5.48	0.10
	Equation		y = 107.4X+89.90			

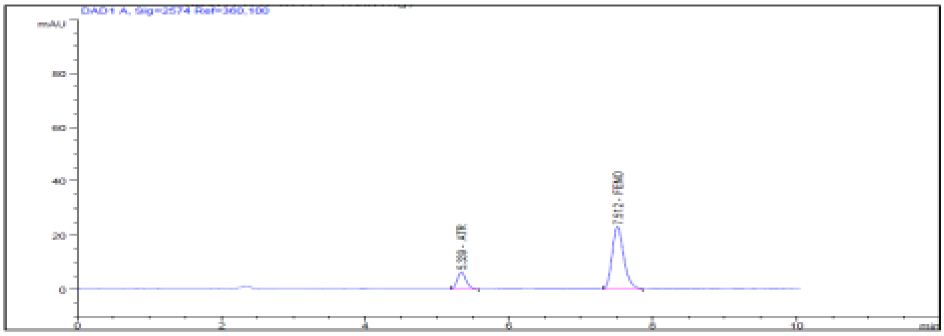

Fig 2:- Chromatogram of standard Atorvastatin and Fenofibrate.

Linearity:

Linearity was established by least squares linear regression analysis of the calibration curve. The correlation coefficient (R2) was calculated, and it was between 0.998-1.0 .The results are shown in **Table 2**. The concentration was proportional to the area, and the response of the detector was determined to be linear over the range for Atorvastatin and Fenofibrate was found to be 1-5 µg/mL and 16-80 µg/mL (**Fig. 3 and 4**). Limit of detection and quantification were found to be 0.5132 and 0.3110 µg/mL for Atorvastatin and 0.9682 and 2.934 µg/mL.

Table No 2: Linearity data for Fenofibrate

Method	Concµg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
	10	1058.73	1059.73	1059.23	0.71	0.07
	20	2160	2140.79	2150.39	13.58	0.63

RP-HPLC Method	30	3161.74	3159.17	3160.46	1.82	0.06
	40	4147.68	4222.58	4185.13	52.96	1.27
	50	5140.77	5141.96	5141.37	0.84	0.02
	Equation		y = 101.9x + 79.61			
	R^2		0.999			

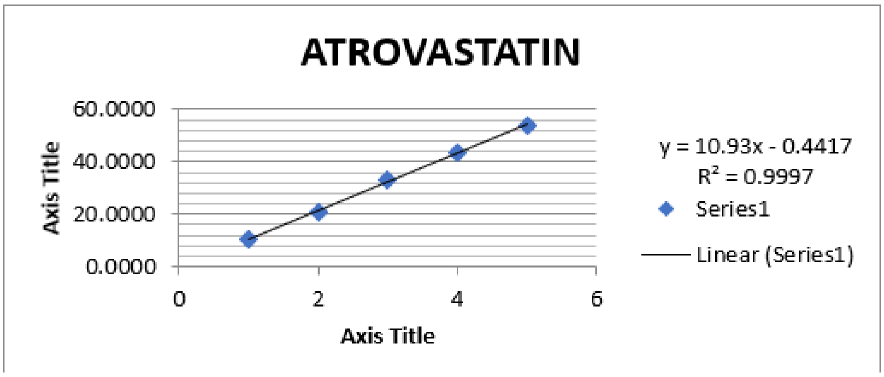

Fig 3. Calibration curve of Atorvastatin

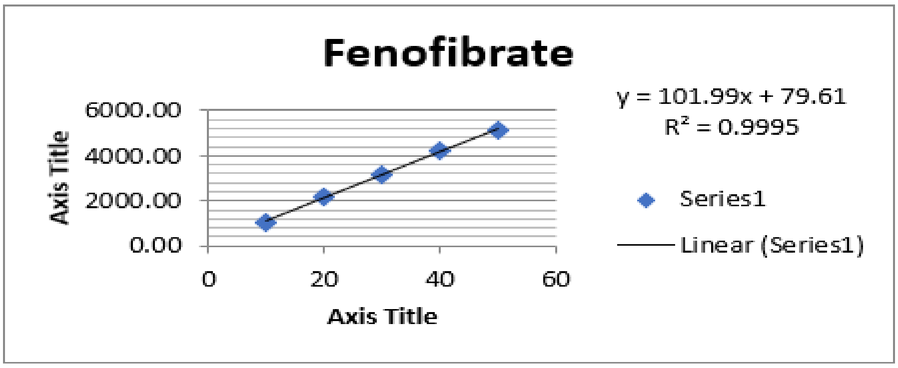

Fig.No.4: Calibration curve of Fenofibrate

Accuracy:

Accuracy was studied by recovery study using standard addition method. The accuracy was determined by measuring the recovery of Atorvastatin and Fenofibrate at three different levels (80,100, and 120 %). The % recovery was 98-102% and % RSD was found to be less than 2. Results of recovery studies are shown in **Table 3**, which specified that the method was highly accurate for simultaneous determination of both the drugs.

Table.3. Statistical Validation of Recovery Studies Atorvastatin and Fenofibrate

METHOD	Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation*	% RSD
Rp-HPLC Method	80%	ATR	100.33	0.2673	0.2664
		FENA	100.68	1.15	1.14
	100%	ATR	99.80	0.072	0.072
		FENA	100.61	0.1871	0.1860
	120%	ATR	99.67	0.18	0.18
		FENA	100.8603	0.292	0.2899

Robustness:

This study showed % RSD less than 2, concerning % assay for both the drugs which indicate that the method developed is robust. The results are shown in **Table 4 and 5**.

Table No.4 Result of Robustness Study of Atorvastatin

Parameters	Conc. (µg/ml)	Amount of detected(mean ±SD)	%RSD
Chromatogram of flow change 0.9ml	5	54.55±0.45	0.53
Chromatogram of flow change 1.1 ml	5	56.91±0.30	0.83
Chromatogram of comp change 74 MEOH +26 WATER	5	71.0±0.13	0.18

Chromatogram of comp change 76 Methanol+ 24 WATER	5	72.50±0.49	0.68
Chromatogram of comp change wavelength change 256 nm	5	47.3±0.08	0.16
Chromatogram of comp change wavelength change 258 nm	5	56.94±0.10	0.17

Table No.5. Result of Robustness Study of Fenofibrate

Parameters	**Conc.(µg/ml)**	**Amount of detected (mean ±SD)**	**%RSD**
Chromatogram of flow change 0.9ml	80	4141.75±1.68	0.04
Chromatogram of flow change 1.1 ml	80	4223.34±3.95	0.09
Chromatogram of comp change 74 MEOH +26 WATER	80	4123.55±2.79	0.07
Chromatogram of comp change 76 Methanol+ 24 WATER	80	4181.2±1.67	0.04
Chromatogram of comp change wavelength change 256 nm	80	4151.04±1.29	0.03
Chromatogram of comp change wavelength change 258 nm	80	4125.38±1.65	0.04

Precision:

The precision of the proposed method was tested by performing repeatability (intra-day) and intermediate precision (inter-day) studies. The precision data is summarised in the **Table 6**, revealed that the % RSD was less than 2 and there by indicating that the method was sufficiently precise.

Table No .6 Result of Intraday and Inter day Precision studies on RP-HPLC for Atorvastatin and Fenofibrate

METHOD	**Drug**	**Conc^{n}(µg/ml)**	**Intraday Precision**		**Interday Precision**	
			Mean± SD	**%Amt Found**	**Mean± SD**	**%Amt Found**
Rp-HPLC METHOD	**ATR**	20	20.24± 0.26	101.19	20.26±1.42	101.29
		30	30.22±11.53	100.74	30.12±11.73	0.35
		40	40.39±76.73	100.97	40.73±6.29	101.84
	FENA	20	20.07± 34.44	100.34	20.01±22.66	102.73
		30	30.45±11.53	101.49	30.37±6.26	100.05
		40	40.81±59.88	102.02	4260.33±7.09	102.57

System suitability:

The System suitability of the method was studied by analysing the standard and sample solutions initially and at different time intervals. The % recovery of Atorvastatin and Fenofibrate was found to be 100.2 and 100.8.[7]

Assay:

The proposed method was applied for the analysis of marketed tablets and two replicate determinations were performed. The interference of excipients was studied by comparing the chromatograms of standards and formulation. The results of this assay yielded 100.2 % and 99.9 % of the label claim for Atorvastatin and Fenofibrate respectively. The results of this assay indicated that the developed method is selective for the analysis of both Atorvastatin and Fenofibrate in formulations without interference of the excipients.

Forced degradation studies:

Forced degradation studies to evaluate the stability indicating properties and specificity of the method. All forced degradation samples were analysed by using the DAD detector to ensure the homogeneity and purity of Atorvastatin and Fenofibrate. The conditions of stress degradations were optimized with respect to the strength of the reagent and exposure period so as to achieve degradation. Forced degradation studies under acidic condition by using 0.1N HCl and product degradation was found to be 16.07 % and 15.84% for Atorvastatin and Fenofibrate for 1 hr respectively. Degradation behaviour under alkaline condition by using 0.1N NaOH and product degradation was found to be 91.92 % and 11.52 % for Atorvastatin and Fenofibrate for 1 hr. Oxidation degradation was done by using hydrogen peroxide and product degradation was found to be 26.98 % and 9.09 % for Atorvastatin and Fenofibrate for 1 hr respectively. Photolytic degradation was found to be 0.64 % and 3.62 % for Atorvastatin and Fenofibrate respectively. Atorvastatin and Fenofibrate were almost stable in relative humidity stress conditions and showed a minimum degradation. Summary of stress degradation results is given in Table 7 and Fig. 5-9. The advantage of this method from the previous methods proposed for the drugs is that it gives a lesser retention time and also the detection method used is economical and easily available. [7]

Table 7: Stability-indicating method data for ART and FENO

Parameter	ART(% degradation)	FENO% degradation)
Acidic(0.1N HCL for 1hr)	16.07	15.84
Alkaline (0.1N NaH for 1hr)	91.92	11.52

Hydrolytic(HPLC waters for 1hr)	0.64	3.62
Oxidative(3% H2O2 for 1 hr)	26.98	9.09
Photo(sun light for 24 hr)	0.32	0.61

HPLC: High Performance Liquid Chromatography

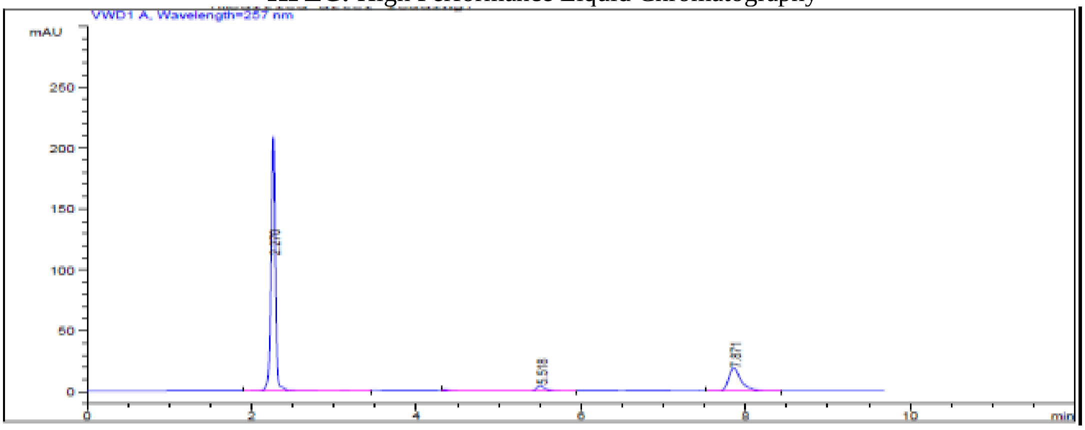

Figure.5 : Chromatogram of ART and FENO degraded with acid hydrolysis

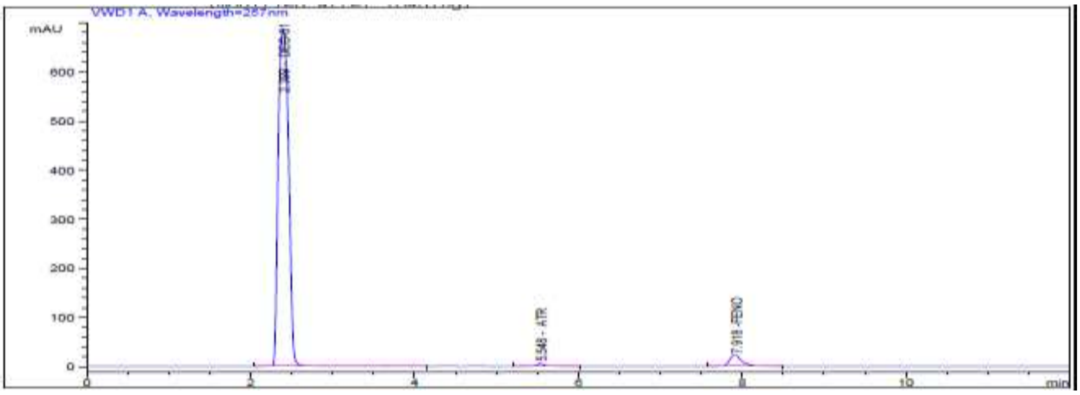

Figure. 6: Chromatogram of ART and FENO degraded with alkali hydrolysis

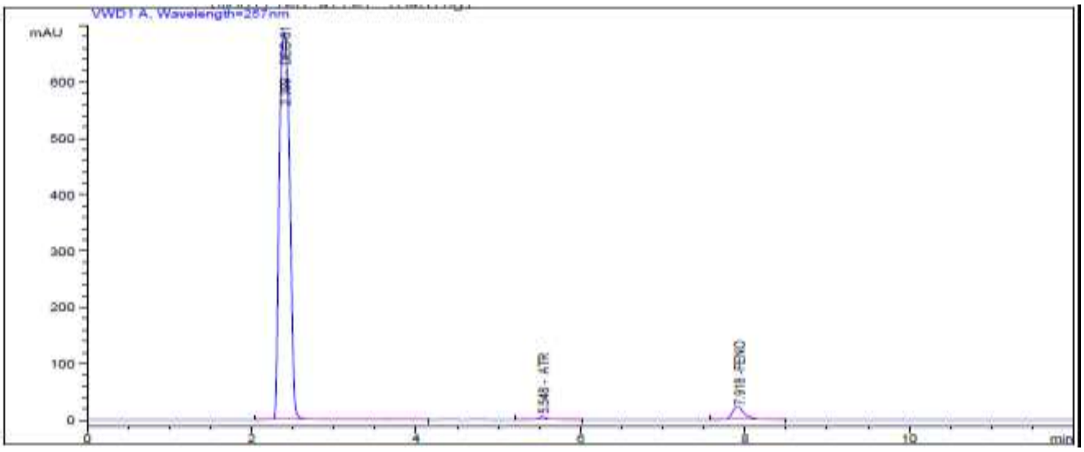

Figure. 7: Chromatogram of mixture of ART and FENO degraded with neutral hydrolysis

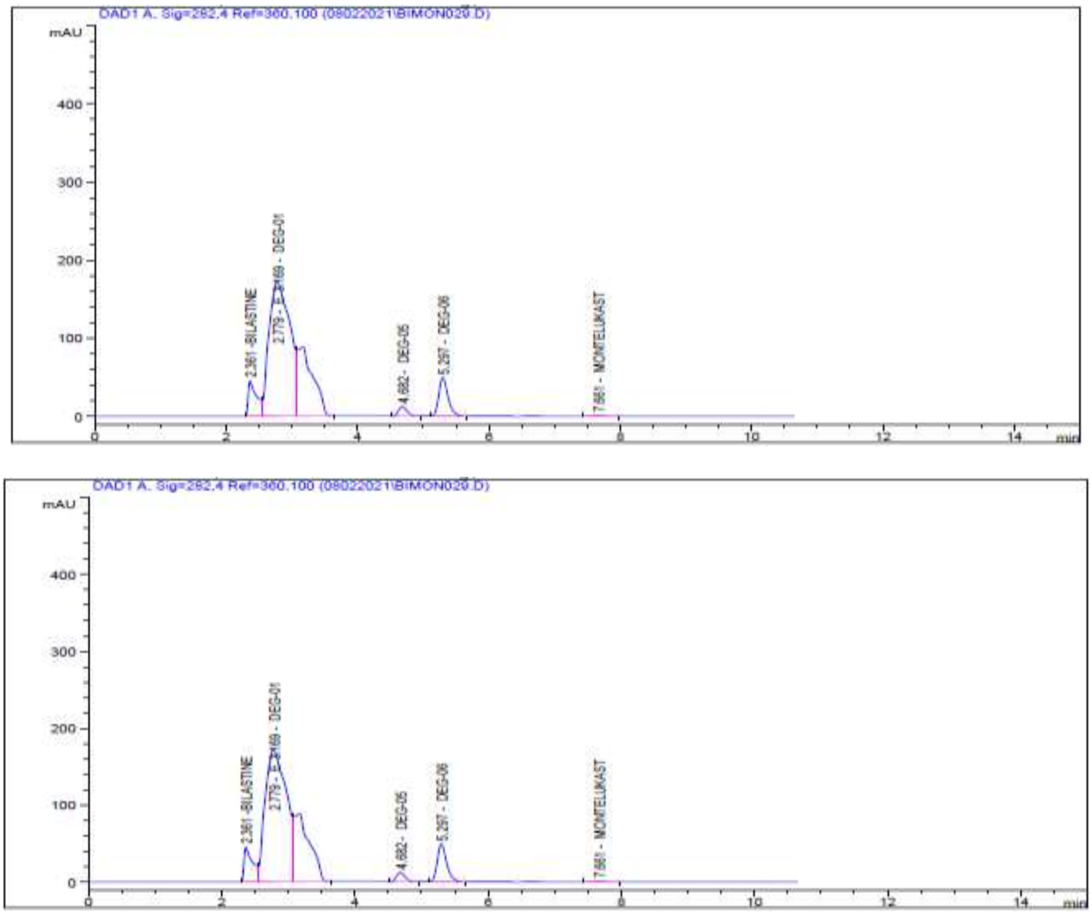

Figure. 8: Chromatogram of ART and FENO degraded with oxidative hydrolysis

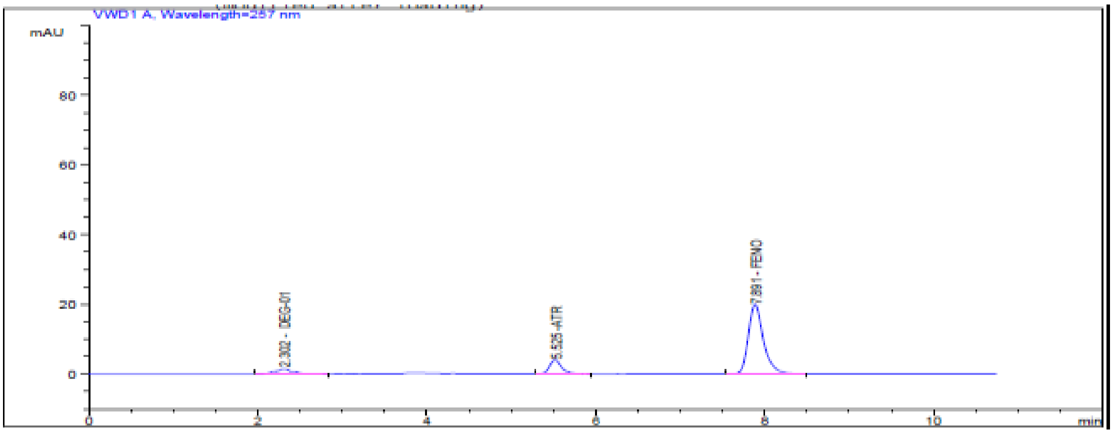

Figure. 9: Chromatogram of ART and FENO degraded with exposed to direct sunlight

CONCLUSION

The study concludes that Atorvastatin and Fenofibrate are most labile to oxidative stress conditions but stable enough to acid, alkaline and photolytic stress conditions. The developed RP-HPLC method was simple, reliable, economical, and found to be more precise, stability indicating, rugged and robust. Validation of this method was accomplished and the results obtained meet all requirements. The method was also found to be highly reproducible with a good accuracy and precision. The results demonstrated that the method would have a great value when applied in routine quality control analysis and stability studies for Atorvastatin and Fenofibrate. [1-7]

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