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# Validated Reverse Phase HPLC Method for Simultaneous Estimation of Atorvastatin and Atenolol in Tablets

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## ABSTRACT

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Atorvastatin and Atenolol in tablets. The compounds were separated on an ODS analytical column with a mixture of Acetonitrile, and Phosphate buffer (pH 4.5±0.05 adjusted with ortho phosphoric acid) in the ratio 72:28 (v/v) as mobile phase at a flow rate of 1.0 mL min<sup>-1</sup> at 238 nm. The retention times of Atorvastatin and Atenolol was found to be 2.167 and 3.737 minutes. Calibration plots were linear over the concentration ranges of 4–20 µg mL<sup>-1</sup> and 20–100 µg mL<sup>-1</sup> respectively. Validation studies revealed the method is specific, rapid, reliable and reproducible. Stability or stress studies was carried out for acidic alkaline, oxidative hydrolysis, thermolytic, relative humidity and photolytic exposure on the drug substance and drug product The high recovery and low relative standard deviation confirm the suitability of the method for determination of atorvastatin and atenolol in tablets.

**Keywords:** Atorvastatin, Atenolol, HPLC, stability studies, tablets.

## INTRODUCTION

Atorvastatin [1] (ATR) is a synthetic lipid lowering agent which inhibits HMG-Co A reductase. Chemically ATR is (β R, δ R)-2-(4-fluorophenyl)- β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1 heptanoic acid, calcium salt (2:1) trihydrate. Atenolol [2] (ATL) is (RS)-4-(2-hydroxy-3-Isopropylaminopropoxy)-phenylacetamide. ATR is not official in any pharmacopoeia while ATL is official with IP, describes a titrimetric method for the determination of ATL, but does not involve simultaneous determination with ATR. Detailed survey of literature for ATR revealed several methods based on different techniques, viz. HPLC,

for its determination in human serum [3]; LC-MS for its determination of its metabolites in serum [4]; HPTLC for its determination in pharmaceuticals [5].

ATL is a  $\beta$ 1-selective (cardio-selective) adrenoreceptor antagonist drug commonly used for management of hypertension, prevention of heart diseases as angina pectoris and control of some forms of cardiac arrhythmia [6]. Several analytical methods have been reported for the determination of atenolol in pharmaceutical formulations. The United States Pharmacopeia (2003) describes a method that uses high performance liquid chromatography (HPLC) with UV detection for assay of atenolol tablets [7]. The method recommended by British Pharmacopoeia (2001) involves UV spectrophotometry [8]. Other methods reported in the literature for the determination of atenolol in pharmaceutical formulations include visible spectrophotometry [9-12], UV derivative spectrophotometry [13], HPLC [14], high performed thin layer chromatography [15-16], potentiometry [17-19], capillary electrophoresis [20-22], and voltametry [23-24].

In recent years pharmaceutical preparations containing both these drugs have been available commercially. Although, many methods for estimation of ATR and ATL individually have been reported in the literature, no single method is available for their simultaneous determination. Because use of this preparation is increasing rapidly, however, it is essential to develop a suitable analytical method for simultaneous estimation of ATR and ATL in pharmaceutical preparations. As HPLC methods have been widely used for routine quality-control assessment of drugs, because of its sensitivity, repeatability, and specificity, we have developed a simple and specific RP-HPLC method for simultaneous determination of ATR and ATL in pharmaceutical dosage forms. According to International Conference on Harmonization (ICH) guidelines analytical methods must be validated before use by the pharmaceutical industry, thus the proposed HPLC–UV method was validated in accordance with, by assessing its selectivity, linearity, accuracy, precision and ruggedness [25].

## MATERIALS AND METHODS

### Chemicals and Apparatus

Atorvastatin and Atenolol were kind gifts from Cadila Health Care Ltd., Bulk Drugs and Ariane Orgachem Pvt.Ltd. Acetonitrile (ACN) of HPLC grade and other reagents of analytical-reagent grade were from Loba Chemicals (Mumbai, India). HPLC-grade water was used for preparation of buffer.

Analysis was performed with a Shimadzu (Japan) chromatograph equipped with an LC-10 AD VP binary solvent-delivery module, an SPD-10A UV–visible detector and Spinchrome software for data handling.

### Chromatographic Conditions

Samples were injected through a Rheodyne injector valve model 7125 with 20- $\mu$ L sample loop. ATR and ATL were separated on a Phenomenex ODS C-18 column (250 mm  $\times$  4.6 mm i.d. 5- $\mu$ m particles) under reversed-phase partition chromatographic conditions. The mobile phase was a mixture of ACN and phosphate buffer (pH 4.5  $\pm$  0.05 adjusted with orthophosphoric acid) in the ratio 72:28 (v/v). The flow rate was 1.0 mL min<sup>-1</sup> and the analytes were monitored at 238 nm.

The equipment was controlled by a PC workstation with Shimadzu CFR chromatography software installed. The system was used in an air-conditioned HPLC laboratory ( $20 \pm 2^\circ\text{C}$ ). Before analysis the mobile phase was degassed by use of a PCI sonicator (PCI analytics, INDIA) and filtered through a  $0.45 \mu\text{m}$  filter (Millipore, Bangalore, India). Sample solutions were also filtered through a  $0.45 \mu\text{m}$  filter. The system was equilibrated before each injection.

### Preparation of the Standard Solutions

Individual stock solutions of ATR and ATL were prepared by dissolving the drugs ( $\sim 10 \text{ mg}$  and  $\sim 50 \text{ mg}$ , accurately weighed) in  $2 \text{ mL}$  of methanol and further diluted to  $100 \text{ mL}$  with mobile phase (final concentration  $100 \mu\text{g mL}^{-1}$  and  $500 \mu\text{g mL}^{-1}$ , respectively).

Mixed standard solution was prepared by accurately weighing ( $\sim 10 \text{ mg}$  and  $\sim 50 \text{ mg}$ , accurately weighed) in  $2 \text{ mL}$  of methanol and further diluted to  $100 \text{ mL}$  with mobile phase (final concentration  $100 \mu\text{g mL}^{-1}$  and  $500 \mu\text{g mL}^{-1}$ , respectively). A  $5 \text{ mL}$  portion was further diluted with the mobile phase to get a concentration of  $10$  and  $50 \mu\text{g mL}^{-1}$  for ATR and ATL respectively. The stock solutions were stored at  $4^\circ\text{C}$  protected from light.

### Calibration Curve

Calibration standards for each analyte were prepared at concentrations of  $4, 8, 12, 16$  and  $20 \mu\text{g mL}^{-1}$  for ATR and  $20, 40, 60, 80$  and  $100 \mu\text{g mL}^{-1}$  for ATL. Separate calibration plots for ATR and ATL were constructed by plotting peak-area against respective concentrations. The correlation coefficient was found to be  $0.9997$  for ATR and  $0.9995$  for ATL.

### Estimation of Atorvastatin and Atenolol in Marketed Formulation

For assay of ATR and ATL in tablets, twenty tablets were weighed and crushed to fine powder, mixed thoroughly. An amount of tablet powder equivalent to  $10 \text{ mg}$  ATR and  $50 \text{ mg}$  ATL was accurately weighed and transferred to a  $100.0 \text{ mL}$  volumetric flask. The mixture was sonicated by shaking with  $2.0 \text{ mL}$  of methanol for  $30 \text{ min}$ , for complete extraction of drugs and volume was made up to mark with mobile phase. The solution was filtered through Whatman filter paper (No.41). A  $5.0 \text{ mL}$  portion of the filtrate was further diluted to  $50.0 \text{ mL}$  with mobile phase, and after equilibration of stationary phase  $20 \mu\text{L}$  of the sample solution was injected for HPLC analysis. The content of ATR and ATL was calculated by comparing the peak area of the sample with that of the standard using the following formula,

$$\% \text{ Label Claim} = \frac{\text{Au} \times \text{W}_{\text{std}} \times \text{Avg.wt}}{\text{As} \times \text{Wt}_{\text{tab}} \times \text{L.C}} \times 100$$

Where,

Au = Peak area of sample, As = Peak area of standard,  $\text{W}_{\text{std}}$  = wt of ATR or ATL in std stock,  $\text{Wt}_{\text{tab}}$  = wt of tablet content, Avg wt = Average weight of tablet, L.C = Label claim of tablet

### Recovery Studies

An accurately weighed quantity of reanalyzed tablet powder was weighed equivalent to ( $\sim 10 \text{ mg}$  of ATR and  $\sim 50 \text{ mg}$  of ATL) and to it ATR and ATL reference standard was added at four different levels, shaken for  $30 \text{ mins}$  in  $2 \text{ ml}$  of methanol and volume made upto the mark with the

mobile phase. The solution was filtered through Whatman filter paper (No.41). A 5.0 mL portion of the filtrate was further diluted to 50.0 mL with mobile phase to get the final concentration. After equilibration of stationary phase 20  $\mu$ L volume of the sample solution was injected separately.

### **Stress Degradation Studies**

An accurately weighed quantity of reanalyzed tablet powder was weighed equivalent to (~10 mg of ATR and ~50 mg of ATL) were transferred to a series of 50.0 mL volumetric flasks. The solutions were kept under the following different conditions:

- *Reflux for 6 h after addition of 25 ml of 0.5M NaOH (Alkali)*
- *Reflux for 6 h after addition of 25 ml of 0.5M HCl (Acid)*
- *Reflux for 6 h after addition of 25 ml of 6% H<sub>2</sub>O<sub>2</sub> (Oxide)*
- *Exposed to 60°C for 24 h (Thermal)*
- *Exposure to UV light for 24 h at 254.0 nm*
- *Exposure to 75% humidity conditions for 24 h*
- *Exposure to sunlight for 6 h*

After exposure to different stress conditions samples were diluted as described under marketed formulation. Solutions were injected separately and the content of ATR and ATL was calculated by comparing the peak area of the sample with that of standard.

## **RESULTS AND DISCUSSION**

### **Method Development and Optimization**

Column type, solvent selectivity (solvent type), solvent strength (volume fraction of organic solvent(s) in the mobile phase, additive strength, detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so the peak from the first eluting compound did not interfere with those from the solvent or excipients. Other criteria, viz. time required for analysis, appropriate  $k$  range ( $1 < k < 10$ ) for eluted peaks, assay sensitivity, and use of the same solvent system for extraction of drug from formulation matrices during drug analysis, were also considered. After each change of mobile phase the column was re-equilibrated with new mobile phase.

To investigate the appropriate wavelength for simultaneous determination of ATR and ATL, solutions of these compounds in the mobile phase were scanned by UV-visible spectrophotometer (Shimadzu, Japan; model UV-1700) in the range 200–400 nm. Solutions of each substance in the mobile phase were also injected directly for HPLC analysis and the responses (peak area) were recorded. It was observed there was no interference of the mobile phase or baseline disturbance at 238 nm. It was, therefore, concluded that 238 nm is the most appropriate wavelength for analysis of these two substance with suitable sensitivity.

The optimized chromatographic conditions used were of the C<sub>18</sub> column with ACN and phosphate buffer (pH 4.5  $\pm$  0.05 adjusted with orthophosphoric acid) in the ratio 72:28 (v/v) as mobile phase at 1.0 mL min<sup>-1</sup>. This method was therefore validated in accordance with ICH guidelines.

**System Suitability**

System suitability was evaluated by replicate (n=7) injection of the same standard solution containing ATR and ATL at 10 and 50  $\mu\text{g mL}^{-1}$  respectively. The RSD (%) of retention time, peak area, number of theoretical plates and tailing factor for both analytes were within 2%, indicating the suitability of the system (Table 1). The number of theoretical plates and the USP tailing factor were within the acceptance criteria of >2000 and  $\leq 1.5$  respectively, indicating good column efficiency and optimum mobile phase composition.

**Table 1 Results of System Suitability Parameters**

Drug	A.U.C (mv)	$\pm$ SD	Retention Time	Resolution	Asymmetry	Th.Pl/Column	Th.plt/mt
ATR	317.212	0.81	2.167	-----	1.515	2609.83	52690.05
ATL	251.338	0.39	3.737	9.803	1.229	3650.16	73331.0

*a.u.c* area under curve (milli volts), *mean  $\pm$  standard deviation*, *theoretical plates/column (th.plt/column)*, *theoretical plates/meter (th.plt/meter)*

The method was applied for the simultaneous estimation of ATR and ATL in their combined dosage form. The results are recorded in Table 2. The % estimation of the drugs was found to be 99.97% for ATR and 99.89% for ATL representing accuracy of method.

**Table 2 Results of Estimation of Atorvastatin and Atenolol in Marketed Formulation**

Drug	Peak Area	% Label Claim*	$\pm$ SD	% RSD
ATR	249.363	99.97	0.55	0.52
ATL	317.7126	99.89	0.25	0.25

*\*mean of five observations*

**Validation of Proposed Method**

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method. Results are shown in Table 3. CV was found to be  $\leq 2.0\%$  for both the drugs.

**Table 3 Results of Recovery Study**

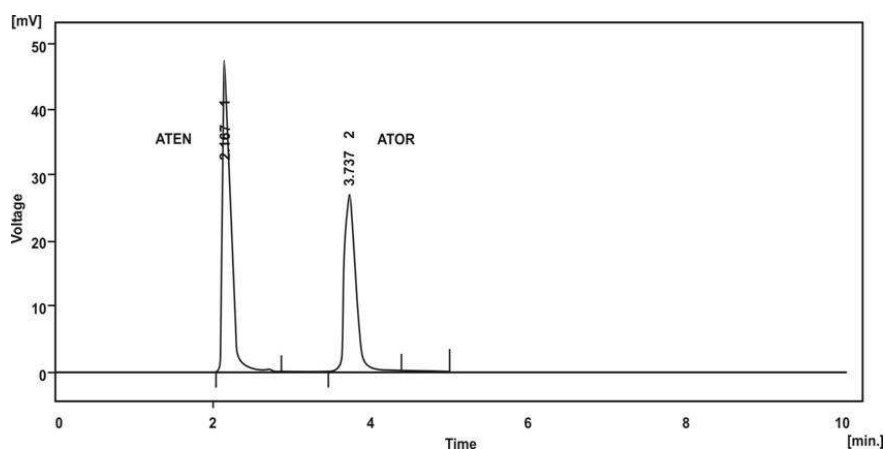
Amount. of Pure drug added (mg)		Amount Recovered (mg)		% Recovery	
ATR	ATL	ATR	ATL	ATR	ATL
4.01	19.99	3.96	20.02	99.17	100.41
6.01	29.98	5.94	29.96	99.02	99.65
8.02	39.98	7.95	39.99	99.47	99.99
10.02	49.90	10.04	50.18	100.98	100.98
<b>*Mean<math>\pm</math>SD</b>				<b>99.66</b>	<b>100.25</b>
<b>CV</b>				<b>0.79</b>	<b>0.57</b>
<b>CV</b>				<b>0.78</b>	<b>0.57</b>

*\*mean  $\pm$  standard deviation (SD), coefficient of variance (CV), \*mean of four observations*

Precision was ascertained by replicate analysis of homogenous samples of tablet powder. Assay precision was expressed as the relative standard deviation (RSD, %), found to be  $\leq 2.0\%$  for both the drugs. Intra-day precision were determined by replicate analysis (n= 3) of the QC samples on the same day; inter-day precision were determined by replicate analysis of the solutions on 1<sup>st</sup>, 3<sup>rd</sup> and on 5<sup>th</sup> day.

Linearity was tested in the concentration range 4–20.0  $\mu\text{g mL}^{-1}$  for ATR and 20–100  $\mu\text{g mL}^{-1}$  for ATL. Separate calibration plots for ATR and ATL were constructed by plotting the peak-area against the respective concentrations and the method was evaluated by determination of the correlation coefficient and intercept.  $R^2$  values  $>0.999$  and intercepts very close to zero confirmed the good linearity of the method. Linearity was studied by taking tablet powder equivalent to 80, 90, 100, 110, 120% of label claim of ATR and the correlation coefficient was found to be 0.9998 for ATR and 0.9991 for ATL.

The specificity of the method was assessed by comparing the chromatograms obtained from drug standards and from marketed formulation indicating the method is selective and specific in relation to the excipients (Fig. 1)



**Fig. 1 Chromatogram of Standard ATL and ATR**

The interference from inactive ingredients was investigated through recovery studies using the standard addition method. This procedure was carried out by adding known amounts of ATR and ATL reference substance into pre-analyzed tablet powder.

Ruggedness studies was carried out by different analyst, interday and intraday variation. The CV for analyst to analyst variation was found to be 0.39 for ATR and 0.27 for ATL. The CV for intraday and interday was found to be 0.36 for ATR and 0.49 for ATL and 1.58 (ATR) and 1.57 (ATL) respectively.

Robustness of the proposed method was ascertained by deliberately changing the mobile phase pH, detection wavelength and flow rate of the mobile phase. The results were found to be well within the limits as shown in Table 4.

Further, an intentional degradation was performed. Blank solutions were used during the analysis and both reference substance and drug product solutions were subjected to degradation. Solutions containing 0.5 mg mL<sup>-1</sup> of acid, base and H<sub>2</sub>O<sub>2</sub> (as per ATL concentration) were used.

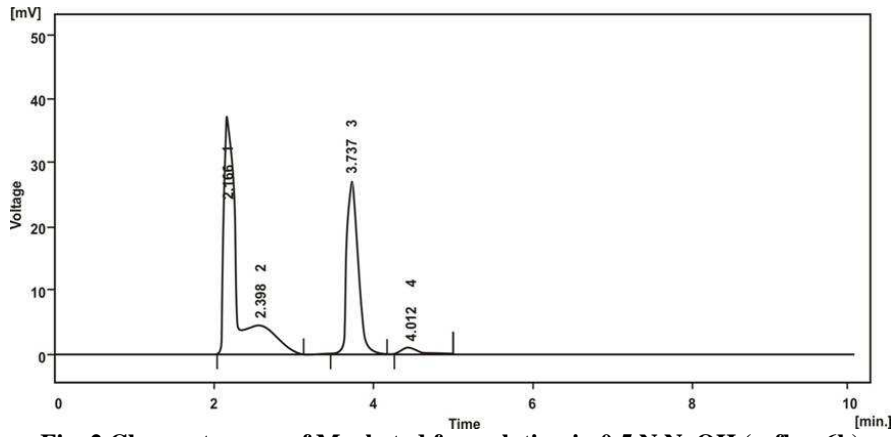
**Table 4 Results of robustness study**

Deliberate Changes in parameters	System suitability parameters				% Label Claim	
	ATR		ATL		ATR	ATL
	Rt	Assymetry	Rt	Assymetry		
Change in Wavelength (254.0 nm)	2.162	1.558	3.741	1.225	99.13	99.04
Flow rate (0.8 ml/min)	1.741	1.525	2.995	1.242	98.97	99.52
Flow rate (1.2 ml/min)	2.604	1.510	4.489	1.211	100.37	99.72
Mobile Phase pH 4.3	2.102	1.592	4.012	1.441	98.84	99.07

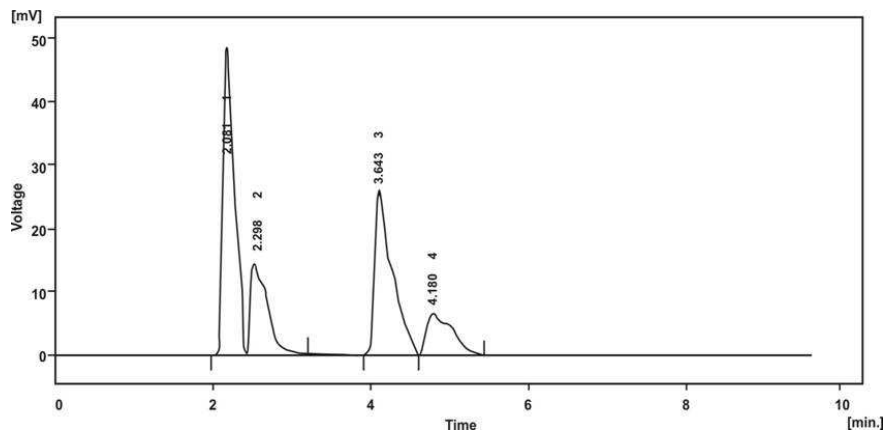
Following the degradation period, all samples were prepared for analysis as previously described and chromatographed (Fig 2-8). Results are shown in Table 5. The results indicate that the drugs were found to be susceptible to degradation in all most all conditions. The ATL and ATR showed generation of one additional peaks under alkaline, acidic and humidity conditions. However the peak distortion was observed under thermal and photolytic stress for ATR

**Table 5 Results of Estimation under Specificity Study**

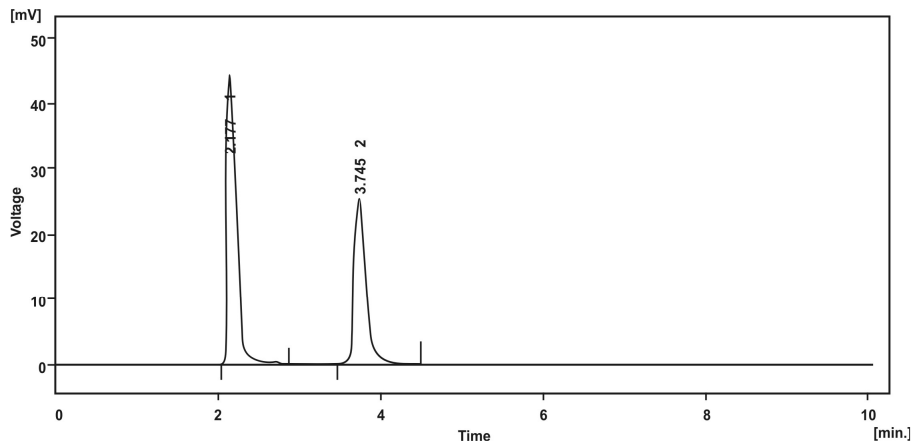
Sample (Treated)	% Un-degraded		Rt (Degraded product)	
	ATR	ATL	ATR	ATL
Reflux for 6 hrs with 0.5 NaOH	94.92	92.26	4.01	2.39
Reflux for 6 hrs with 0.5 N HCl	96.36	91.68	4.10	2.30
Reflux for 6 hrs with 6% H <sub>2</sub> O <sub>2</sub>	97.51	98.02	--	--
Exposed 60°C for 24 hrs	98.79	97.91	--	--
Exposure to UV Light for 24 hrs at 254.0 nm	97.17	98.84	--	--
Humidity (75%)	97.56	97.69	4.12	2.41
Exposure to Sunlight for 6 hrs	96.49	96.71	--	--



**Fig. 2** Chromatogram of Marketed formulation in 0.5 N NaOH (reflux 6h)

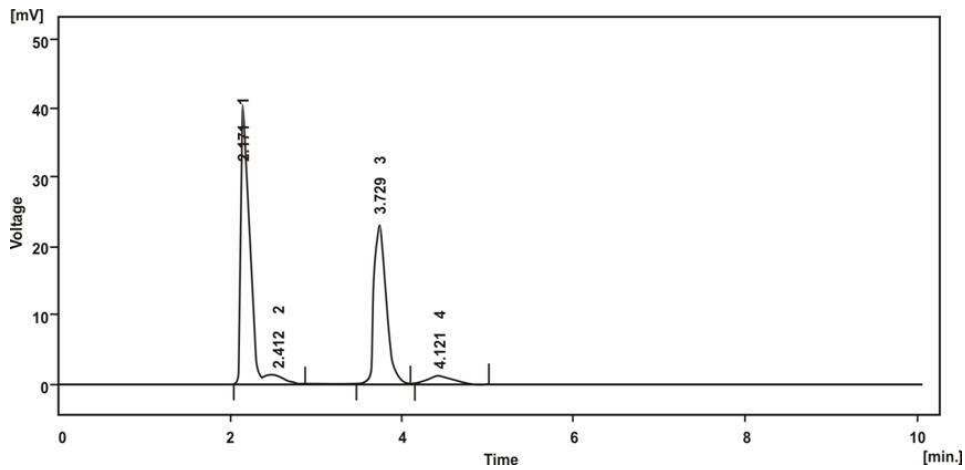


**Fig. 3** Chromatogram of Marketed formulation in 0.5 N HCl (reflux 6h)

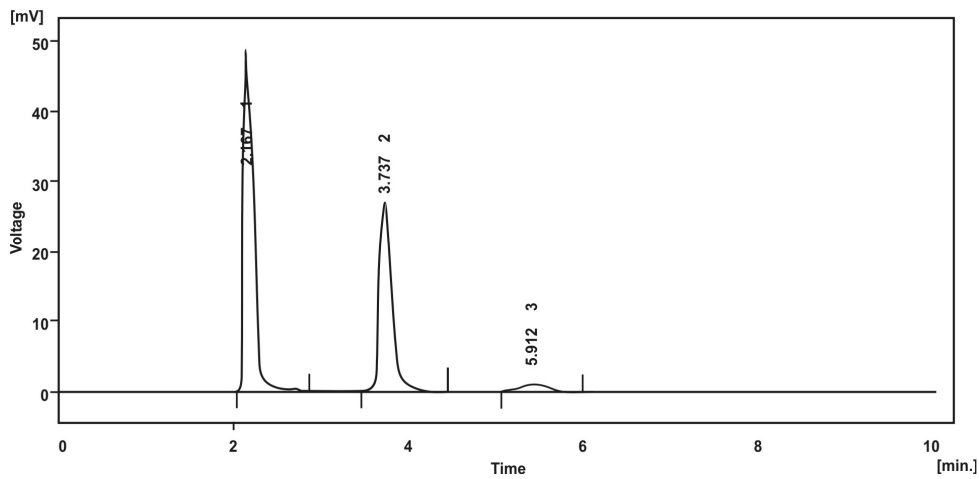


**Fig. 4** Chromatogram of Marketed formulation in 6% H<sub>2</sub>O<sub>2</sub> (reflux 6h)

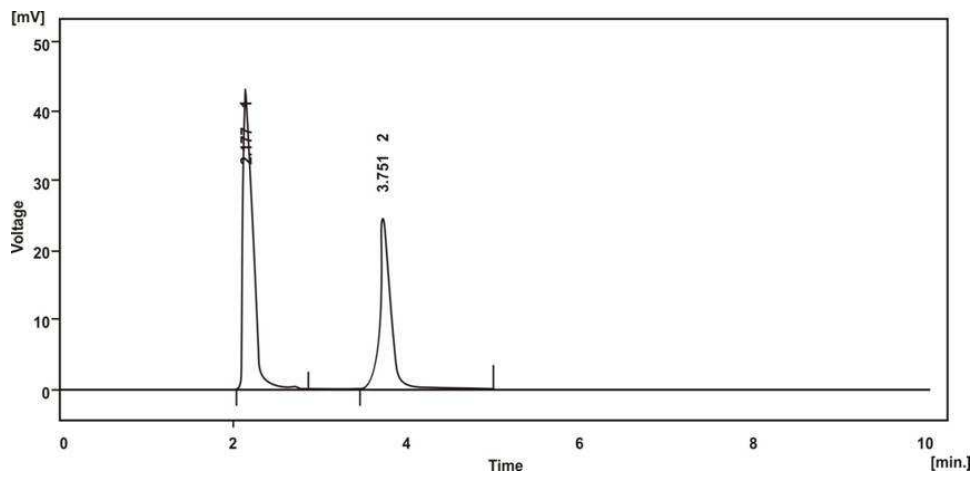




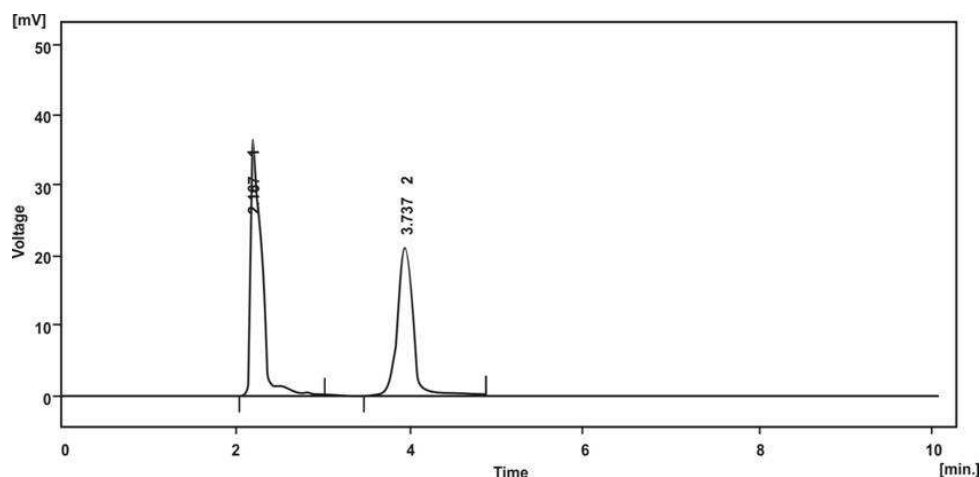
**Fig. 5** Chromatogram of Marketed formulation to Humidity (75%)



**Fig. 6** Chromatogram of Marketed Formulation exposed to sun light 6h



**Fig. 7** Chromatogram of Marketed Formulation exposed to 60°C (24h)



**Fig. 8 Chromatogram of Marketed Formulation under UV light exposure (24h)**

Limit of detection for ATR and ATL was found to be 0.0125 $\mu$ g/mL and 0.0625 $\mu$ g/mL respectively.

### CONCLUSION

The results obtained by RP-HPLC method for determination of Atovastatin and Atenolol are reliable, accurate and precise. The method does not require prior separation of one drug from another. Hence, it can be employed for routine quality control analysis of ATR and ATL in their combined dosage form. The proposed method is less time consuming so can be successfully applied for the dissolution analysis of the two drugs, estimation from the biological fluids. The method can be used as stability indicating method for the estimation of two drugs in presence of their degradation products.

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