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A Stability-Indicating LC Method for the Simultaneous Determination of Metoprolol, Atorvastatin and Ramipril in Combined Pharmaceutical Dosage Form

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ABSTRACT

A simple, rapid, and precise method is developed for the quantitative simultaneous determination of metoprolol (ME), atorvastatin (AT) and ramipril (RA) in combined pharmaceutical dosage form. A chromatographic separation of the three drugs was achieved with a Hypersil C8, 15-cm analytical column using buffer–acetonitrile (55:45 v/v). The buffer used in mobile phase contains 0.02 M sodium perchlorate in double distilled water. The instrumental settings are flow rate of 1.0 mL min^{-1}, column temperature at ambient, and detector wavelength of 210 nm for ME, AT and RA using a ultra violet detection. Methanol is used a diluent. The resolution among ME, AT and RA were found to be more than 2.0. Theoretical plates for ME, AT and RA were >2500. Tailing factor for ME, AT and RA were 1.20, 1.00 and 1.20. ME, AT and RA and their combination drug product were exposed to acid, base, neutral, thermal, photolytic, hydrolytic and oxidative stress conditions, and the stressed samples were analysed by the proposed method. Peak homogeneity data of ME, AT and RA is obtained using photodiode array detector, in the stressed sample chromatograms, demonstrated the specificity of the method for their estimation in presence of degradants. The described method shows excellent linearity over a range of 40–600 µg mL^{-1} for ME, 16–240 µg mL^{-1} for AT and 8-120 µg mL^{-1} for RA. The correlation coefficient for ME, AT and RA are found greater than 0.999. The relative standard deviation for six measurements in three sets of each drug in tablets was always less than 2.0%. The proposed method was found to be suitable and accurate for quantitative determination and the stability study of ME, AT and RA in pharmaceutical preparations.

Keywords Column liquid chromatography; Capsule dosage forms; Method validation and quantification; Pharmaceutical preparation; Metoprolol succinate; atorvastatin calcium; ramipril.

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INTRODUCTION

Metoprolol (ME) [Bis[(2*RS*)-1-[4-(2-methoxyethyl)phenoxy]-3-[(1- methylethyl) amino]propan-2-ol] butanedioate]. Beta-adrenoceptor antagonist. Atorvastatin (AT) [[*R*,(*R**,*R**)]-2-(4-fluorophenyl)-b,d-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl] -1 **H**pyrrole-1-heptanoic acid as the calcium salt] a synthetic lipid- lowering agent which is about a 100 times as potent as the other drugs in its class and at lower costs than most of the others. AT is an inhibitor of 3-hydroxy- 3-methylglutaryl-coenzyme A reductase. Ramipril (RA) [[2S,3aS,6aS]-1-[(2S)-2-[[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl] octa hydro cyclopenta pyrrole-2-carboxylic acid [1] is a highly lipophilic, long acting ACE inhibitor. Angiotensin converting enzyme inhibitor. The drug is used for treating blood pressure and congestive heart failure. It effectively reduces both supine and standing blood pressure without significant alteration in the pulse rate. Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light and enables recommendation of storage conditions, retest periods and shelf life to be established [2]. The two main aspects of drug products that play an important role in shelf life determinations are assay of active drug and degradants generated during the stability study. Literature survey revealed several analytical methods such as spectrophotometry, spectrofluorimetry, simple and stability indicating TLC, simple and stability indicating LC, Raman spectroscopy, LC-MS-MS and LC-ESI-MS have been reported for the determination of AT, RA and ME in pharmaceutical dosage forms and biological samples [3-22].

Stability-indicating methods have been reported for assays of various drugs in drug products containing only one active drug substance. Only few stability indicating methods are reported for the assay of combination drug products containing two or more active drug substances. The objective of this work was to develop an analytical LC procedure, which would serve as stability indicating assay method for combination drug products of ME , AT and RA.The literature survey reveals that several methods were reported for the individual estimation, also for double combinations (AT+RA, RA+ME, ME+AT) and also one tribal combination (AT+RA+AS). But in this publication explains only the analysis of commercial analysis not given forced degradation. None of the reported analytical procedures describe a method for simultaneous determination of ME, AT and RA in combined pharmaceutical dosage form. If the reported individual methods are applied for the analysis of the tablets containing ME, AT and RA it would require thrible time for analysis, and the method would not be rapid, less expensive or economical, whereas the simultaneous determination of the ingredients of the tablets would save analysis time and also economy. In the present study attempts were made to develop a rapid, economical, precise and accurate method for the simultaneous determination of the ingredients of this combination in the presence of their degradants.

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EXPERIMENTAL

Chemicals and Reagents

ME, AT and RA standards were obtained from Dr.Reddy's Laboratories Ltd (Hyderabad, India). The LC grade Acetonitrile were purchased from Merck Fine Chemicals, Mumbai, India. The AR grade sodium lauryl sulphate, ortho phosphoric acid, sodium hydroxide (NaoH), hydrochloric acid (HCl) were purchased from Merck Fine Chemicals (Mumbai, India) and hydrogen peroxide (H_2O_2) was from Qualigens Fine Chemicals, Mumbai, India and high pure water prepared by using Millipore Milli Q plus purification system The 0.45µm-Pump nylon filter was obtained from Advanced Micro Devices Pvt. Ambala Cantt, India. Hypersil C8, column was procured from Thermo Electron Corporation.

Instrumentation

The combination product of ME, AT and RA label claim (50+20+10, 50+10+5,25+10+2.5)mg respectively. The LC system used for method development and validation was from Waters (Milford, USA) and equipped with a 2695 separation module with inbuilt auto injector and 2996 photodiode array detector. The output signal was monitored and processed using Empower software (Waters) on a Pentium computer (Digital Equipment Co) (Laboratory B).

Preparation of Standard and Sample Solution

Mixed Standard Solution was prepared by weighing 42 mg of metoprolol succinate, transferring 10 mL of atorvastatin standard stock solution about concentration 1.6 mg mL^{-1} and 5 mL of ramipril standard stock solution 1.6 mg mL^{-1} into a 100 mL volumetric flask and diluted with diluent. The standard solution contained 420 µg mL^{-1} of metoprolol succinate and 160 µg mL^{-1} of atorvastatin and 80 µg mL^{-1} of ramipril.

Sample preparation was prepared by open and transfering carefully ten capsules content into a 250 mL volumetric Flask. To this flask, 150 mL of diluent were added, and the solution was sonicated for 30min with intermittent shaking, maintained sonicator temperature at 25°C,followed by shaking of 15 mints. Then the volume was made up with diluent and centrifuged at 3000 rpm for 5 min. The centrifuged solution filtered through a 0.45-µm filter. From the filtered solution, 5 mL were transferred into a 25 mL volumetric flask and diluted to volume with diluent.

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RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions

The solubility of ME as drug substance was white crystalline powder freely soluble in water and soluble in methanol. AT calcium is a white to off white powder is insoluble in aqueous solutions of pH 4 and below, slightly soluble in distilled water, acetonitrile and ethanol and freely soluble in methanol, chloroform and dimethylsulphoxide. Known and unknown impurities have similar properties with AT calcium as well. RA was white crystalline powder sparingly soluble in water, freely soluble in methanol. Hence methanol is used as a diluent and the chromatographic elution was carried out in acetonitrile than in methanol due to poor selectivity.

To develop the stability-indicating method different stationary phases like C18, C8, CN different mobile phases containing buffers like phosphate, ammonium acetate and ortho phosphoric acid with different pH (3–5) and organic modifier (acetonitrile) were used. Our objective of the chromatographic method development was to achieve a peak tailing factor <2.0, retention time in between 3min to 15min, along with a resolution among metoprolol, atorvastatin and ramipril >2.0. The chromatographic separation was achieved using an Zorbax C8 column (150 · 4.6 mm i.d.). Changing the composition of mobile phase optimized the chromatographic method. Resolution between ME, AT and RA was observed on any C8 or CN column but it was difficult to separate both drug degradants on these columns. From the development studies, it was determined that 4.0 mM sodium Lauryl sulphate and 1.0 ml of ortho phosphoric acid in water and acetonitrile in the ration of 55:45 (v/v), had a mobile phase flow rate of 1.0 mL min^{-1} and a column temperature of 40°C. The analytes of this combination had adequate retentions, peak shape, less tailing, more resolution and the chromatographic analysis time was less than 25 min. But retention time consistency is not observed due to Sodium lauryl sulphate buffer, so we changed the mobile phase as 20mM with an adjustment of pH 4.0 with ortho phosphoric acid and little bit changed the chromatography parameters. In optimized conditions ME, AT and RA their degradants were well separated. Typical retention times of ME, AT and RA were about 5.8, 9.2 and 12.5 min. Resolution among ME, AT and RA was found to be greater than 5.0. **Fig – 1.**

Method Validation results of the method

Precision

The precision of the method was studied by determining the concentrations of each drug in the tablets six times. The assay %RSD for ME, AT and RA were 0.1, 0.2 and 0.2. The results of the precision study indicate that the method is reliable (% RSD < 2.0).

Intermediate Precision (Reproducibility)

Intermediate precision of the method was determined by analyzing the samples six times on different days, by different chemists, by using different analytical columns of the same make and different LC systems. The percentage assay was calculated using calibration curves. The assay results of chemist 1 for ME, AT and RA were 101.4, 100.7 and 100.8. The assay results of chemist 2 for ME, AT and RA were 99.4, 99.1 and 99.4.

Linearity

The linearity of an analytical procedure is its ability (with in a given range ie 10% - 150% sike levels) to obtain test results, which are directly proportional to the concentration of the analyte in the sample. The calibration curve solutions contained 42–630 µg mL^{-1} of ME, 16–240 µg mL^{-1} of AT and 8–120 µg mL^{-1} of RA.

Robustness

The robustness of a method is the ability to remain unaffected by small changes in parameters. To determine robustness (tailing factor, %RSD) of the method, experimental conditions were purposely altered. To study the effect of flow rate on the tailing factor of ME, AT and RA it was changed to 0.2 units from 0.8 to1.2 mL min_1. The effect of column temperature was studied at 20 and 30°C instead of 25°C, while other mobile phase components were held constant. The effect of mobile phase composition ME, AT and RA was studied at organic phase composition (acetonitrile) 90% – 110%. At all conditions the tailing factor, %RSD among ME, AT and RA were not more than 2.0.

Determination of Limit of Quantification and Limit of Detection (LOQ and LOD)

The LOD and LOQ for ME, AT and RA were determined at a signal to- noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. The LOD values for ME , AT and RA were 2.3 µg mL^{-1} ,0.2 µg mL^{-1} and 0.4 µg mL^{-1}, and the LOQ values were 7.7 µg mL^{-1} , 0.8 µg mL^{-1} and 1.3 µg mL^{-1} respectively for 10 µL injection volume.

Accuracy (Recovery Test)

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at five levels, 25, 50, 75, 100 and 150% of the label claim of the tablet (50 mg of metoprolol succinate, 20 mg of atorvastatin and 5 mg of ramipril). Placebo equivalent to ten capsules were transferred into a 250 mL volumetric flask, and the amounts of ME, AT and RA drug substance at 25, 50, 75, 100 and 150%of the label claim of the tablet were added. The recovery samples were prepared as per the procedure mentioned in sample preparation. Three samples were prepared for each recovery level. The solutions were then analysed, and the

percentage recoveries were calculated from the calibration curve. The recovery values for ME, AT and RA ranged from 97.6 to 102.8%, 97.7 to 101.1% and 97.6 to 101.5%. The average recovery of five levels (fifteen determinations) for ME, AT and RA were 100.32% (2.1), 99.58% (1.2) and 99.78 (1.6). %RSD shown in parenthesis.

Results of validation parameters, system suitability parameters and regression analysis of calibration curves values are shown in **Table 1, 2, 3**.

Solution Stability

The stability of the standard and test solution was tested at intervals of 24 and 48 h. The stability of solutions was determined by comparing results of %assay of ME, AT and RA. The %assay values were within 3.0 upto 48 h. The results indicate that the solutions were stable for 48 h at ambient temperature as there was no formation of any unknown peak and solution remained stable.

Procedure for Forced Degradation

Study of Forced degradation of each drug product was carried out under thermolytic, Humidity, photolytic, acid, base, hydrolytic and oxidative stress conditions. The ICH guideline [23-24] state the minimum desired exposure as 200 Wh/m2, which corresponds to a change in absorbance of 0.5 AU of quinine actinometer at 400 nm. This change was observed in 24 h of irradiation. A second photolytic stress test experiment with greater irradiation time was in 48 h.

Acidic Degradation

Sample preparation was prepared by open and transferred ten capsules content into a 250 ml volumetric flask, 10ml of 0.1N HCl added and the mixture kept reflux for 30 min at 60°c .The solution was allowed to attend ambient temperature, then it was neutralized with 0.1N NaOH to pH 7 150 mL of diluent was added, and the solution was sonicated for 30min with intermittent shaking, maintained sonicator temperature at 25°C, followed by shaking of 15 mints. Then the volume was made up with diluent and centrifuged at 3000 rpm for 5 min. The centrifuged solution filtered through a 0.45-µm filter. From the filtered solution, 5 mL were transferred into a 25 mL volumetric flask and diluted to volume with diluent.

Alkali Degradation

Sample preparation was prepared by by open and transferred ten capsules content into a 250 ml volumetric flask, 10ml of 0.1N NaoH added and the mixture kept reflux for 30 min at 60°c .The solution was allowed to attend ambient temperature, then it was neutralized with 0.1N HCl to pH 7 150 mL of diluent was added, and the solution was sonicated for 30min with intermittent shaking, maintained sonicator temperature at 25°C, followed by shaking of 15 mints. Then the volume was made up with diluent and centrifuged at 3000 rpm for 5 min. The

centrifuged solution filtered through a 0.45-µm filter. From the filtered solution, 5 mL were transferred into a 25 mL volumetric flask and diluted to volume with diluent.

Oxidative Degradation

Sample preparation was prepared by open and transferred ten capsules content into a 250 ml volumetric flask, 10ml of 5.0% H_2O_2 added and the mixture kept reflux for 30 min at 60°c .The solution was allowed to attend ambient temperature, then it was solution was sonicated for 30min with intermittent shaking, maintained sonicator temperature at 25°C, followed by shaking of 15 mints. Then the volume was made up with diluent and centrifuged at 3000 rpm for 5 min. The centrifuged solution filtered through a 0.45-µm filter. From the filtered solution, 5 mL were transferred into a 25 mL volumetric flask and diluted to volume with diluent.

Thermal Degradation

About 2.0 g of drug product blend were kept at 80°C for 24 h. Then the solution was prepared to achieve 400 µg mL^{-1} of ME, 160 µg mL^{-1} of AT and 80 µg mL^{-1} of RA.

Humidity Degradation

About 2.0 g of drug product blend were kept at 90% RH for 168 h. Then the solution was prepared to achieve 400 µg mL^{-1} of ME, 160 µg mL^{-1} of AT and 80 µg mL^{-1} of RA.

UV-Short Degradation (254 nm)

About 2.0 g of drug product blend were exposed to UV short light for 24 h. Then the solution was prepared to achieve 400 µg mL^{-1} of ME, 160 µg mL^{-1} of AT and 80 µg mL^{-1} of RA.

Sunlight Degradation (366 nm)

About 2.0 g of drug product blend were exposed to UV short light for 24 h. Then the solution was prepared to achieve 400 µg mL^{-1} of ME, 160 µg mL^{-1} of AT and 80 µg mL^{-1} of RA.

Results of percentage degradation of ME, AT and RA at different stressed conditions were shown in **Table – 4, 5, 6**. Respective chromatograms of different stressed conditions were shown in **Fig – 2.**

CONCLUSION

The isocratic RP-LC method developed for the analysis of binary mixtures of ME, AT and RA in their pharmaceutical preparations is precise, accurate and with a short run time. The method was fully validated showing satisfactory data for all the method validation parameters

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tested. The developed method is stability-indicating, separates degradants and can be conveniently used by the quality control department to determine the assay of pharmaceutical preparations and also stability samples.

Table-1. Summary of validation parameters for metoprolol succinate, atorvastatin calcium, and ramipril by the proposed LC method

Parameters	ME	AT	RA
LOD (μg mL^{-1})	2.3	0.2	0.4
LOQ (μg mL^{-1})	7.7	0.8	1.3
Accuracy (%) ± % RSD	100.32 ± 2.1	99.58 ± 1.2	99.78± 1.6
Precision (% RSD)			
Inter-day (n = 2)	0.2 - 1.0	0.4 - 1.4	0.9 – 3.7
Repeatability (% RSD)	0.1	0.2	0.2

Table- 2. System suitability test parameters for for metoprolol succinate, atorvastatin calcium, and ramipril by the proposed LC method (n = 6)

Parameters	ME	AT	RA
Retention time (min) ± % RSD	5.99 ± 0.15	8.75 ± 0.44	1.75 ± 0.30
Tailing factor ± % RSD	1.23 ± 0.10	1.08 ± 0.15	1.20 ± 0.10
Theoretical plates ± % RSD	14761.20 ± 0.66	2340.21 ± 0.95	11638.24 ± 0.47

Table- 3. Regression analysis of the calibration curves for metoprolol succinate, atorvastatin calcium, and ramipril by the proposed LC method

Parameter	ME	AT	RA
Slope	10517.050	37033.350	17876.187
Intercept	27281.78	-4623.41	44084.24
Correlation coefficient (r)	0.999	0.999	0.999

Table -4: Results of analysis of forced degradation study samples using proposed method, indicating percentage degradation and peak purity angle of Metoprolol peaks in chromatograms

Stress Condition	Drug Product-Metoprolol		
	% Assay	Purity angle	Purity threshold
Acid degradation	98.7	0.116	0.258
Base degradation	98.7	0.123	0.259
Peroxide degradation	97.8	0.117	0.260
UV degradation	100.7	0.122	0.258
Heat degradation	98.4	0.119	0.258
Sunlight degradation	99.5	0.126	0.259
Water degradation	97.4	0.117	0.259
Humidity degradation	100.2	0.123	0.259

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Table-5: Results of analysis of forced degradation study samples using proposed method, indicating percentage degradation and peak purity angle of Atorvastatin peaks in chromatograms

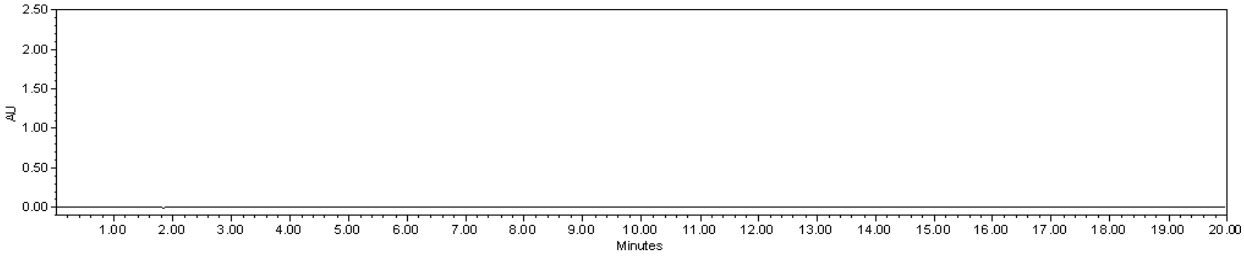
Stress Condition	Drug Product-Atorvastatin		
	% Assay	Purity angle	Purity threshold
Acid degradation	98.9	0.055	0.247
Base degradation	100.9	0.121	0.249
Peroxide degradation	96.2	0.042	0.249
UV degradation	100.8	0.0385	0.249
Heat degradation	96.2	0.036	0.248
Sunlight degradation	99.7	0.034	0.249
Water degradation	99.4	0.040	0.249
Humidity degradation	101.1	0.035	0.249

Table-6: Results of analysis of forced degradation study samples using proposed method, indicating percentage degradation and peak purity angle of Ramipril peaks in chromatograms

Stress Condition	Drug Product-Ramipril		
	% Assay	Purity angle	Purity threshold
Acid degradation	96.2	0.055	0.247
Base degradation	96.6	0.168	0.329
Peroxide degradation	98.5	0.178	0.344
UV degradation	100.2	0.174	0.325
Heat degradation	94.7	0.165	0.345
Sunlight degradation	99.1	0.216	0.350
Water degradation	97.2	0.150	0.336
Humidity degradation	101.4	0.179	0.339

Fig –1 Typical HPLC Chromatogram of (a) Blank, (b) Sample, (c) Standard

(a)Chromatogram of Blank



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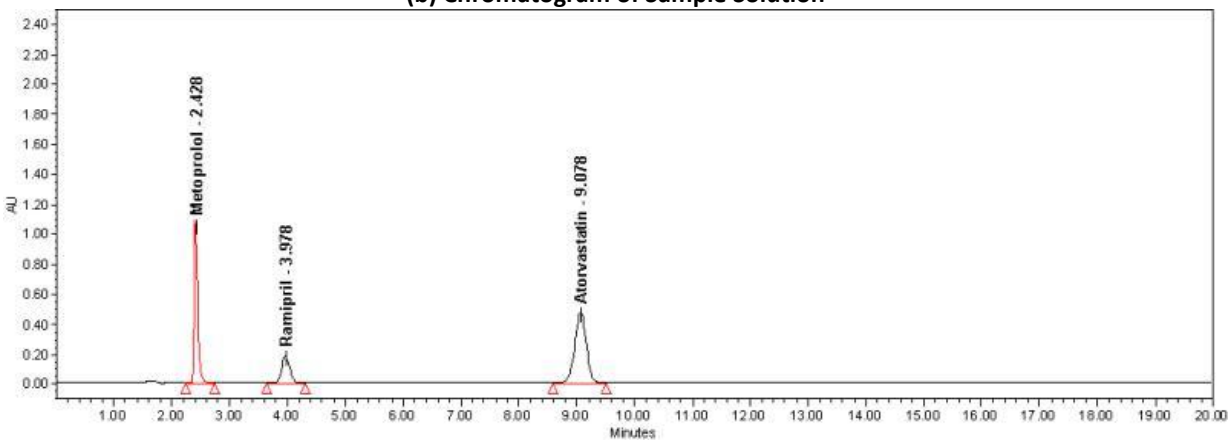
(b) Chromatogram of Sample Solution



(c) Chromatogram of Standard Solution

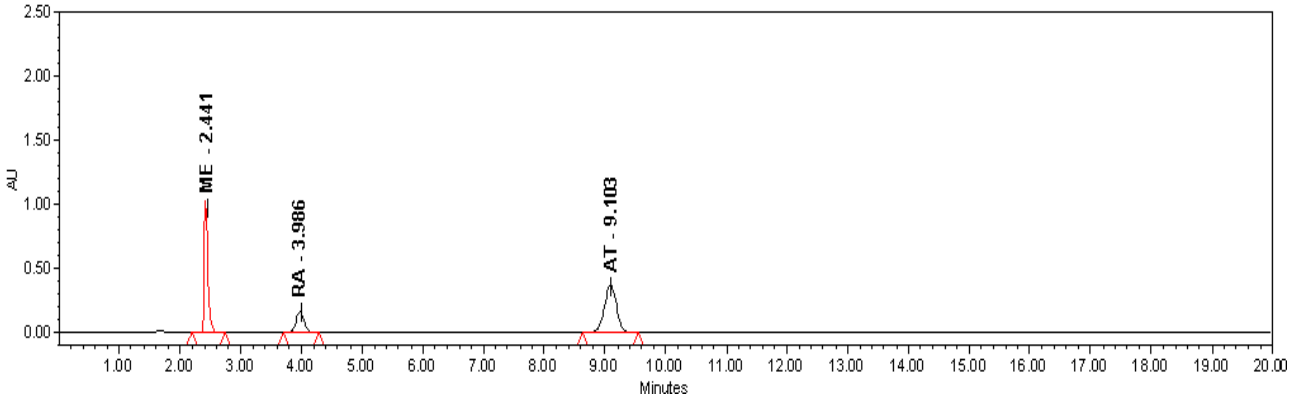
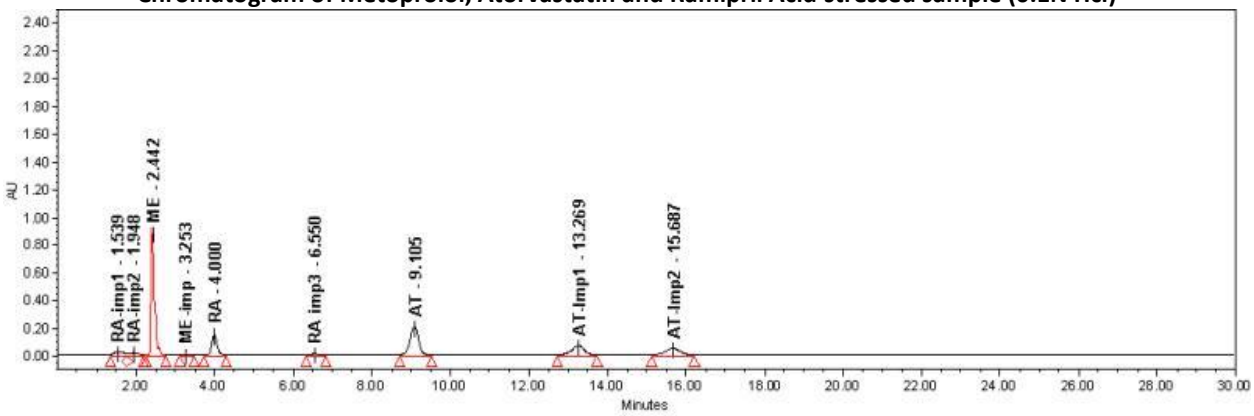


Fig. 2. Typical HPLC chromatograms recorded during forced degradation studies.
Chromatogram of Metoprolol, Atorvastatin and Ramipril Acid stressed sample (0.1N Hcl)



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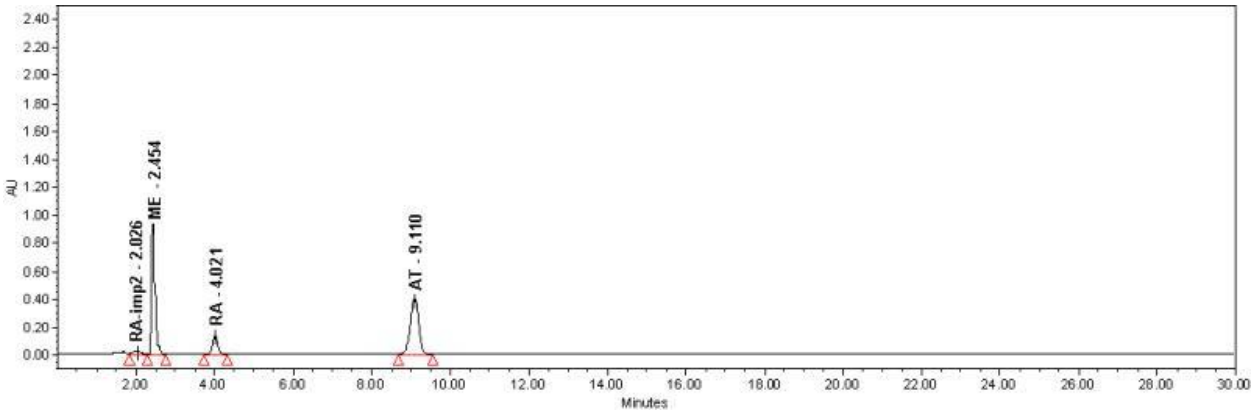
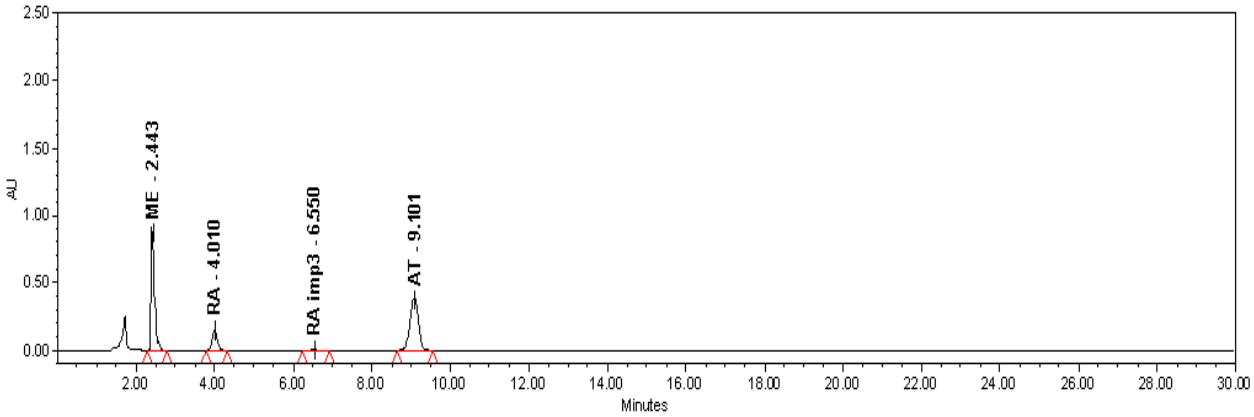
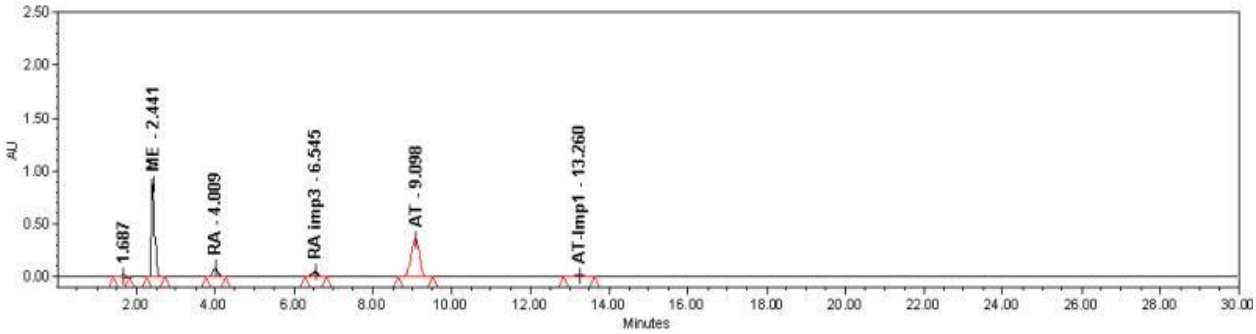
Chromatogram of Metoprolol, Atorvastatin and Ramipril base stressed sample (0.1N NaOH)



Chromatogram of Metoprolol, Atorvastatin and Ramipril Peroxide stressed sample (5% H_2O_2)



Chromatogram of Metoprolol, Atorvastatin and Ramipril heat stressed sample (80^{0}C for 24 hrs)



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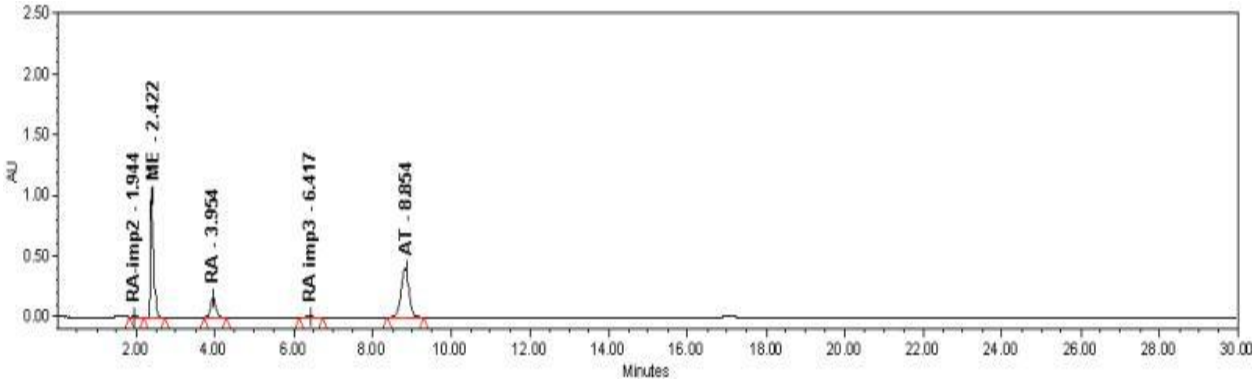
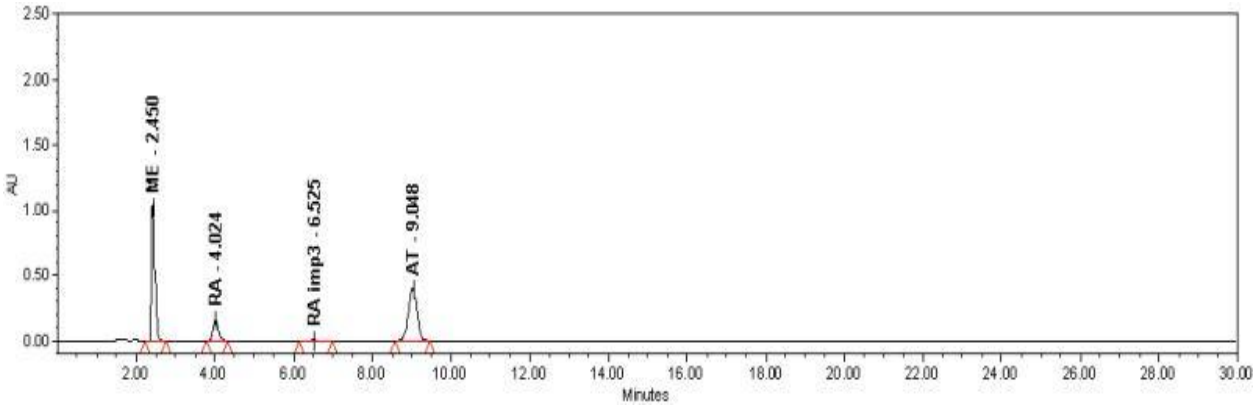
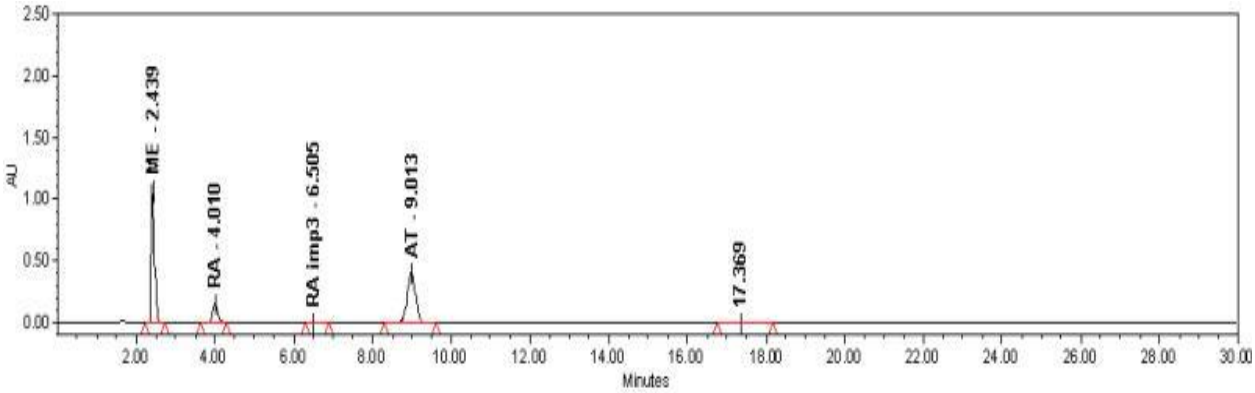
Chromatogram of Metoprolol, Atorvastatin and Ramipril humidity stressed sample (90% for 168hrs)



Chromatogram of Metoprolol, Atorvastatin and Ramipril UV stressed sample (254nm for 24hrs)



Chromatogram of Metoprolol, Atorvastatin and Ramipril UV stressed sample (366nm for 24hrs)



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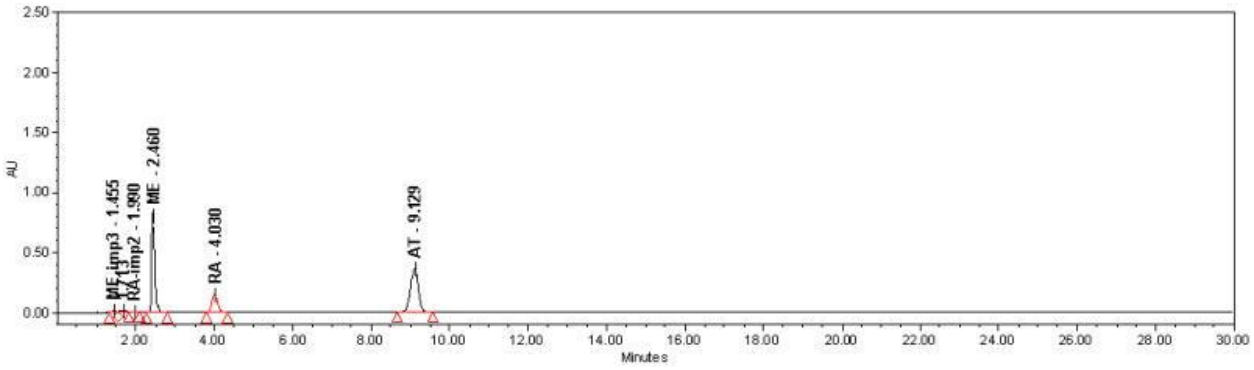
Chromatogram of Metoprolol, Atorvastatin and Ramipril WATER Stressed sample



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