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Method Development and Validation for the Estimation of Ramipril and Atorvastatin in Pharmaceutical Formulation by RPHPLC

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Research Articles

Method Development and Validation for the Estimation of Ramipril and Atorvastatin in Pharmaceutical Formulation by RP-HPLC

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Abstract

Atorvastatin and Ramipril were simultaneously estimated in pharmaceutical dosage forms by developing fast and precise RP-HPLC method. Chromatographic separations on C18 column (4.6mm×250mm, 5µm) were achieved using mixture of 0.1% OPA buffer (pH-3.0): Acetonitrile: Methanol, (45:50:05 v/v). pH adjustment of aqueous phase was done by using 0.5% Triethylamine. The peak response was monitored at 227 nm after injecting the sample into HPLC system at a flow rate of 1.0 ml/min. The calibration curve was linear over the range of Ramipril 10-30µg / ml and Atorvastatin 20-60µg / ml. Atorvastatin's average RT was 9.2826±0.0107 and Ramipril was 4.548±0.0366. Atorvastatin's percentage recovery value is 99.47% and Ramipril's 98.50% which confirms the excipients do not interfere in the formulation. Thus, the suggested HPLC technique can therefore be used for the simultaneous determination of these two drugs in pharmaceutical dosage forms due to speed, simplicity and high accuracy.

Key words: Atorvastatin, Ramipril, RP-HPLC

Introduction

Atorvastatin is chemically referred to as [R-(R*, R*)]-2-(4-fluoro- phenyl)- ß-δ- dihydroxy-5-(1-methylethyl) -3- phenyl 4-[(phenylamino) carbonyl]-1H- pyrrole-1- hepatonoic acid[6]. It is a competitive inhibitor of HMG-CoA reductase, a lipid lowering agent and used in hyper-cholestrolemia. Ramipril is chemically referred to as (2s,3as, 6as)- 1- [(s)-2- [(s)-1-(ethoxycarbonyl)-3-phenyl propyl] amino propanoyl] octahydrocyclopenta[6] pyrrole-2- carboxylic acid. It is an angiotensin converting enzyme drug and used in ranovascular hypertension[7]. The present work describes the development and validation of the RP-HPLC method that can quantify the drugs from the combined forms of dosage simultaneously. For an individual determination of Atorvastatin and Ramipril, few chromatographic methods have been defined. The aim of the present work was to develop and validate an accurate technique for Atorvastatin and Ramipril to be determined simultaneously. The method was validated in accordance with ICH guidelines[8].

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Methodology

Instrumentation: YoungLin ACME900 HPLC system with C-18 column (4.6 mm ×250mm, 5µm) was used for the present study. Detection of the eluted components was achieved using UV Detector and digital pH meter was used to check the solution's pH.

Reagents and Chemicals

Torrent Pharmaceutical Pvt. Ltd. Baddi provided pure samples of Atorvastatin and Ramipril. The commercial sample of RAMIPTOR capsules (A to Z Life Sciences,) containing 10mg Atorvastatin and 5mg Ramipril were purchased from local pharmacy. Water, methanol, acetonitrile (HPLC grade), Orhtophosphoric

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acid (85-88%) and Triethylamine were obtained from Loba Chemie Pvt. Ltd. Mumbai.

Preparation of stock solutions

Stock solution was prepared by dissolving 100 mg of each drug into 100 ml volumetric flasks comprising 100 ml of methanol individually and 10-60 mcg/ml of working concentration of both drugs were prepared.

Chromatographic conditions

In the present study, the 1% OPA buffer (pH-3.0): Acetonitrile: Methanol, (45:50:05 v/v) are used as mobile phase. pH adjustment of aqueous phase was done by using 0.5% Triethylamine and then using 0.2 µ membrane filter, solution was filtered. Finally all the solutions were degassed prior to use. The column temperature was kept at $\pm 25^{0}$ C. Following injection of the sample into the HPLC system at a flow rate of 1.0 ml / min, the peak response was monitored at 227 nm.

Recommended standard graph method

Following a systematic and comprehensive research on various parameters concerned, the pure sample and dosage forms of Atorvastatin and Ramipril used the following operation and conditions for simultaneous estimation. Before the drug solution was injected, using mobile phase the column was saturated for atleast 45 mins. Atorvastatin (10 mg) and Ramipril (5mg) were accurately weighed and transferred to volumetric flask of 100 ml and dissolved in methanol. The quantity of Atorvastatin 100 mcg/ml and Ramipril 50mcg/ml was produced upto the mark with methanol. From this different dilution of Ramipril 10-30 mcg/ml and Atorvastatin 20-60 mcg/ml with 20 mcg/ml using caffeine as internal standard were made and AUC was determined. The calibration curve was then plotted by ratio of AUC of drug by internal standard *vs* concentration.

Assay of Atorvastatin and Ramipril in formulations

The contents of the twenty Ramiptor capsules were weighed accurately and the average weight was calculated. The equivalent of 10 mg Atorvastatin and 5 mg Ramipril were taken and dissolved in methanol in a volumetric flask of 100 ml. The final concentration of Atorvastatin 100 mcg/ml and Ramipril 50 mcg/ml was produced with methanol. From this different dilution of Ramipril (10-30 mcg/ml) and Atorvastatin (20-60 mcg/ml) were made and AUC was determined using caffeine as internal standard. Finally, sonicated the solution for 3 min, filtered through Whatmann filter paper and lastly diluted the filtrate to final concentration with solvent.

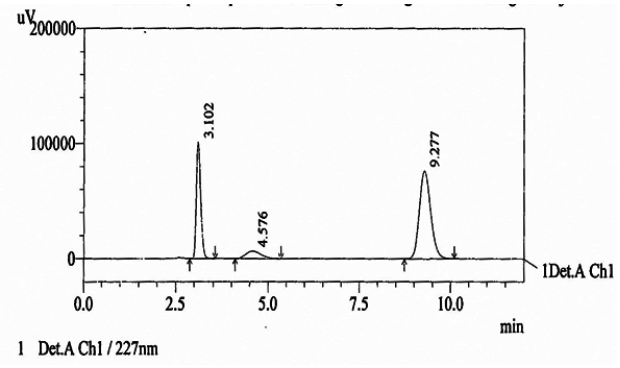

Figure 1: Typical Chromatogram

Validation of developed HPLC Method

Linearity: For Atorvastatin in the concentration range of 20-60 mcg/ml and for Ramipril 10-30 mcg/ml, the standard curve was achieved. The linearity of this technique was assessed utilizing the least square technique using linear regression analysis.

Precision

Precision of the method was determined in terms of repeatability, inter-day and intra-day precision. For each of the middle concentrations, six injections were given from the linearity range and then the proportion of RSD was calculated. The variation in the peak region of Atorvastatin and Ramipril drug dilution was calculated in terms of coefficient of variation.

Accuracy: The precision of the HPLC technique was evaluated with the addition of known quantities of drugs i.e. 80 percent and 120 percent of the middle concentration from the linearity plot was selected and spiked in pre-analyzed drug solution. The samples were then analyzed by the proposed HPLC technique in triplicates and % Relative Standard Deviation and recovery was determined.

Limit of detection (LOD) and limit of quantification (LOQ): LOD is the drug's minimum concentration that is detectable. The measured reference and test replicates of a sample containing a low drug concentration are used to determine the LOD. LOQ is the lowest concentration at which the analyte cannot be continually identified but quantified as well. The LOQ may be equal to the LOD or at concentration much greater.

Where, σ = SD of lowest concentration sample; m = Slope

Results and Discussion

The method developed for both drugs was evaluated for accuracy, linearity and accuracy and percentage of RSD was calculated. The drug recovery was found to be 98.5 percent and 99.47 percent with less than 2 percent RSD value, respectively. Ramipril LOD and LOQ values were 0.082 **µg** and 0.25 **µg**, Atorvastatin 1.5 **µg** and 1.9 **µg**, respectively. It was found that the linearity of the method is extremely reproducible with R2 value of 0.999. Good linearity was achieved within the concentration range of 20-60 **µg** / ml for Atorvastatin and 10-30 **µg** / ml for Ramipril respectively. Figure 1 shows the typical chromatogram. Atorvastatin and Ramipril retention time was found to be 3.10 and 9.27 min respectively with both peaks being well resolved. The first peak at 3.10 was of Atorvastatin while the second peak at 9.27 was of the Ramipril. The additional peak aprt from these two peaks was observed consistently when the process was repeated and hence it was concluded to be of the mobile phase components or any other ingredient. Number of Theoretical plates was found to be 4376 and 577, respectively for Atorvastatin and Ramipril. Tailing factor was found to be 1.215 and 1.224 for Atorvastatin and Ramipril, respectively. With respect to precision, %RSD was found to be <2% for both the drugs.

Conclusion

The RP-HPLC method was developed to estimate Ramipril and Atorvastatin drugs and this method can be used in the pharmaceutical formulations to estimate these drugs simultaneously.

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Conflict of Interest

No conflict of interest declared by authors.

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