SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND RAMIPRIL BY RP-HPLC AND SPECTROSCOPY

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ABSTRACT
A number of analytical methods were reported for the estimation of atorvastatin and ramipril from their individual dosage forms or in combination with other drugs (Valiyare, 2004; Vachareau and Neirinck, 2000). Here successful reverse phase-high performance liquid chromatographic method and spectroscopic methods developed then validated for the analysis of combined dosage form of atorvastatin and ramipril . Individual λ-max for atorvastatin is 247 n.m and that of ramipril is 208 n.m. They intercept at 215 n.m which is fixed as wavelength for reverse phase-high performance liquid chromatographic method.

Keywords: Atorvastatin, Ramipril, RP-HPLC, intersil-ODS, Acetonitrile,Vierodt's equation.

INTRODUCTION

Capsule containing atorvastatin, categorized for hyper cholestrolemia in combination with ramipril, an anti-hypertensive agent found to be therapeutically very effective in cardiac patients (Anonymous, 1992). This combination is widely started to use in India. A to Z Life Sciences, Pondicherry, India marketed this formulation in the trade name Rampitor capsules, which contain atorvastatin 10 mg and ramipril 5 mg. The local quality analysis requirement is that each capsule should contain a minimum of 90.58% and maximum of 110% of the declared amount of the capsules. Eventhough various analytical methods were reported for the estimation of atorvastatin and ramipril in their individual dosage forms as well as in combination with other drugs , none of the methods reported for simultaneous estimation of both compounds in the mixture by the present developed methods, which are simple,rapid and reliable method of assaying quality control of atorvastatin and ramipril containing capsules simultaneously. We have applied simultaneous equation (Vierodt's equation) (Beckett and Stenlake, 1988) to determine the concentration of each drug in the mixture. Mixtures of known composition were used as standards to minimize errors due to the presence of both components in the solution.

EXPERIMENTAL

Theory

Reverse Phase-high performance liquid Chromato-graphic method (RP-HPLC) generally used to separate small polar to semipolar molecules. In reverse phase, normally using various solvents in the increasing order of their strength are Water: Methanol: Acetonitrile: Tetrahydrofuran etc… The stronger the solvent or solvent mixture, then more quickly will elute an organic compound from a particular column Chaudhari *et al.*, 2007; Stanisz and Kania, 2006). In RP-HPLC, the stationary phase is a hydrophobic ligand chemically bonded onto a particulate support. Degree of separation of one component from another components described by RS. It can be calculated using the formula;

$$Rs = \frac{tr^2 - tr^1}{0.5(w_1 + w_2)}$$

Where tr^1 is the retention time of first peak and W_1= width of first peak. Where tr^2 is the retention time of second peak and W_2 = width of second peak.

In the spectroscopic studies, Beer's law serve as the basis for absorption in absorbing species. Simultaneous estimation is concerned with the determination of two solutes in a solution. When the sample contains two absorbing species(X,Y) each of which absorbs at the λ-max of other, it is possible.

To determine the both drugs by applying the technique of simultaneous equation (Vierodt's equation). The absorptivities of "X" at λ_1 and λ_2 are ax_1 and ax_2. The absorptivities of "Y" at λ_1 and λ_2 are ay_1 and ay_2. The absorbances of diluted sample at λ_1 and λ_2 are A_1 and A_2. Let Cx and Cy be the concentration of X and Y in the diluted sample. Two equations constructed based on the fact that, the absorbance of the mixture is sum of the individual absorbance of 'X" and "Y".

At λ_1 $A_1 = ax_1bcx + ay_1bcy$ (1)

At λ_2 $A_2 = ax_2bcx + ay_2bcy$ (2)

For measurement in 1 c.m. cell, b=1, so the equation (2) can be rearrange as;

$$Cy = \frac{A_2 - ax_2Cx}{Ay_2}$$

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Substituting the CY in equation(1) and rearrangement gives;

$$Cx = \frac{A_2ay_1 - A_1ay_2}{Ax_2ay_1 - ax_1ay_2} \quad (3)$$

$$Cy = \frac{A_1ax_2 - A_2ax_1}{Ax_2ay_1 - ax_1ay_2} \quad (4)$$

Thus concentration of "X" and "Y" can be determined.

Instrumentation

Gradient shimadzu-LC-2010 CHT with PDA detector have been used for HPLC analysis.

An Elico-SL 164 double beam UV-visible spectro-photometer with 1 c.m quartz cell have been used for measuring absorbance.

Materials

Various chemicals used are Atorvastatin (reference standard), Ramipril (reference standard), Rampitor capsules which contain atorvastatin 10 mg and ramipril 5 mg. Various solvents used include methanol-HPLC grade, Acetonitrile-HPLC grade, Sodium perchlorate AR and Phosphoric acid AR. Common solvent methanol used for dissolving chemicals in spectroscopic studies.

Methods

After many trial and error, the ideal solvent system for eluting atorvastatin and ramipril is found to be a mixture of 50% acetonitrile and 50% buffer. Buffer have been prepared by incorporating 1 gm sodium perchlorate in 500 ml milli-Q-water, adjusted the pH to 2.5 with dilute phosphoric acid. Flow rate was 1.2 ml/minute. Column temperature was 40^{o}c. Column used was Intersil ODS (250x46 m.m) 5µ. Injection volume was 10 µl.

To prepare standard stock solution 16.66 mg of atorvastatin and 8.4 mg of ramipril weighed and transferred into 25 ml standard flask and dissolved in 15 ml methanol, then sonicated and made up the volume. 10 ml of this is transferred to 50ml standard flask and volume made up with mobile phase. Thus the concentration of atorvastatin is 0.13328mg/ml and that of ramipril is 0.0672mg/ml in the analyte solution.

To prepare sample stock solution, 10 capsules weighed, crushed into powder and an equivalent weight of 25 mg weighed and transferred into 25 ml standard flask, added 15 ml methanol, then sonicated and made up the volume with methanol. From this 10 ml of clear solution after centrifugation diluted to 50 ml with mobile phase to get 200µg/ml concentration. So the sample concentration is 0.2mg/ml of analyte solution. 10 ml of each standard and sample solutions were injected into HPLC system for five times, retention time and average peak areas were recorded. The amount of drug present in pharmaceutical formulation calculated through peak area ratio of content to that of standard calibration curve.

For spectroscopic studies, λ-max determined by making reference standard of each drug at a concentration of 10 µg/ml prepared and scanned within the wavelength range of 200-380 nm against the corresponding reagent blank.

Standard stock solutions were prepared by dissolving 25 mg of each drug in 10 ml of methanol, sonicated, then volume made upto 25 ml with methanol to get concentration of 1 mg/ml solution. From the stock solution suitable solutions were made with methanol to get working standard of 6 µg/ml of atorvastatin and 3 µg/ml of ramipril. To construct Beer's plot, different aliquots of atorvastatin (2-10 ml, 1 ml = 10 µg/ml) and ramipril (2-10 ml, 1 ml = 10 µg/ml).

Were taken and diluted to 10 ml with methanol. Mixed standard solutions were prepared from working standard solutions of two drugs, then absorbance of solutions were measured at 247 n.m for atorvstatin and 208 n.m for ramipril, which were found as their respective λ-max . Thus calibration curve constructed.

To prepare sample stock solution, for spectroscopic analysis 10 capsules weighed, crushed into powder and an equivalent weight of 25 mg weighed and transferred into 25 ml standard flask, added 15 ml methanol, then sonicated and made up the volume with methanol. Thus the concentration of sample will be 1 mg/ml of analyte solution.Clear solution from this taken for measuring absorbance at 247 n.m for atorvastatin and 208 n.m for ramipril respectively. The amount of drug present in formulation was calculated using calibration curve.

RESULTS AND DISCUSSION

In the estimation by HPLC % recovery for atorvastatin found to be 99.2% and that of ramipril is 99.6%. In HPLC, the linearity range was found to be 100-500 µg/ml for atorvastatin as well as for ramipril.

In order to validate the developed method, linear fit of the system, precision and accuracy have been checked.

To check linear fit of the system, least square regression analyses were carried out for slope, intercept and correlation coefficient. The method proved to be precise from the peak area ratios obtained by actual determination of six replicates of a fixed amount of drug. RSD for atorvastatin was 0.318% and that of

ramipril was 1.001%. Thus the method found to be very precise. Accuracy of the method have been determined from recovery studies by carrying out estimation in different amounts like 80%, 100% and 120% of bulk sample of atorvastatin and ramipril added to pre-analysed formulation. % recovery studies show RSD 0.035% for 80%. 0.027% for 100% and 0.030 for 120%. Thus the method have been proved to be accurate.

In spectroscopic studies, by applying the measured absorbance in Vierodt's formula, the recovery for atorvastatin is found to be 97.7% and that of ramipril is 100.6%. To validate the developed method precision, accuracy and linearity have been checked. Precision of proposed method was ascertained by actual determination of six replicates of fixed amount of the drug. Results of precision studies showed relative standard deviation (R.S.D.) less than 2% which indicated the method has good reproducibility. To determine the accuracy of proposed method, recovery studies were carried out by taking different amounts (80%, 100% and 120%) of bulk sample of atorvastatin and ramipril within the linearity range were taken and added to pre-analysed formulation. Present recovery values of pure drug from pre-analysed solutions of formulation were inbetween 99.64-100.03% which indicated the method is accurate and also reveals commonly used excipients and additives in the formulation do not made any interference in the developed method.

CONCLUSION

The developed RP-HPLC and spectroscopic methods are found to be extremely simple, rapid, precise and accurate method for assaying commercial atorvastatin and ramipril containing capsules without prior separation. By comparing two methods, HPLC method found to be more reliable. Excipients interference were also negligible. Mobile phase neither corrosive nor deteriorate the column too much. Running time needed for single assay was also less. So by all means, this RP-HPLC method can be applied to assay commercial dosage forms containing ramipril and atorvastatin with good accuracy.

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