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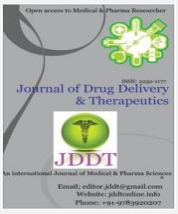

Open Access Research Article

SPECTROPHOTOMETRIC AND REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHODS FOR SIMULTANEOUS DETERMINATION OF ATORVASTATIN AND PIOGLITAZONE IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

Simple, accurate, precise, and sensitive ultraviolet spectrophotometric and reversed-phase high-performance liquid chromatographic (RP-HPLC) methods for simultaneous estimation of Atorvastatin (ATOR) and Pioglitazone (PIO) in combined tablet dosage form have been developed and validated. The spectroscopic methods employs formation and solving of simultaneous equation at 247 nm and 267 nm as 2 wavelengths for estimation of ATOR and PIO respectively (method 1) While method 2 involves formation of Q-absorbance equation at 233 nm (isoabsorptive point) and 267 nm (λ max of Pioglitazone) with methanol as solvent. Beer's law is obeyed in the concentration range of 5.0–50.0 mcg/mL for ATOR and PIO, respectively. The RP-HPLC method uses HPLC system with a Phenomenex Luna C18 (5 µm x 25cm x 4.6mm i.d) using Methanol, acetonitrile and potassium dihydrogen phosphate buffer, pH 2.5 adjusted with orthophosphoric acid (60:20:20 v/v) at a flow rate of 1.0 mL/min at ambient temperature as the mobile phase. The detection was carried out using an ultraviolet detector set at 233 nm. For the HPLC method, Beer's law is obeyed in the concentration range of 5.0-50.0 µg/ml for ATOR and PIO, respectively. LOD values for ATOR and PIO were found to be 57.12µg/ml and 12.01 µg /ml respectively. All the methods have been successfully applied for the analysis of the drugs in a pharmaceutical formulation. Results of analysis were validated statistically and by recovery studies.[1-4]

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INTRODUCTION:

Atorvastatin[R-(R,R)]-2-(4-fluorophenyl)-ß,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4 [(phenylamino) carbonyl] -1H-pyrrole 1-heptanoic acid is a hypolipidemic agent which is used for treatment of hyperlipidemia and Pioglitazone (±)-5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy] phenyl]methyl]-2,4-thiazolidinedione monohydrochloride is a hypoglycemic agent which is used in treatment of hyperglecemia.

A literature survey revealed that different analytical methods involving Specrtrophotometric and column high-performance liquid chromatography (HPLC) for determination of ATOR and PIO in biological fluids and in pharmaceutical preparations have been developed. Because of absence of an official pharmacopoeial method for the simultaneous determination of ATOR and PIO in pharmaceutical formulations, efforts were made to develop an analytical method for estimation of ATOR and PIO in their combined dosage form using HPLC method and UV Spectroscopy.

MATERIALS AND METHODS:

Instruments

A UV-visible (UV-Vis) double beam spectrophotometer (Model 1601; shimadzu) with 1 cm matched quartz cells was used for the spectrophotometric method. For the HPLC method, an HPLC system consisting of pump (Shimadzu LC 10AT VP) with universal loop injector (Rheodyne 7725 i) of injection capacity 20 µL. Detector consists of photodiode array detector (PDA) SPD-10 AVP UV-Visible detector, for separation column used was Phenomenex Luna C18 (5 µm x 25cm x 4.6mm i.d).

Method I: Simultaneous equation method.

This spectrophotometric technique is employed when the two absorbing drugs in the sample X and Y absorbs at the λ max of each other. The standard solution of strength 100 µg/ml of ATOR and PIO were prepared in methanol separately. From overlain spectra of ATOR

(10 µg/ml) and PIO (10 µg/ml) two wavelengths 247nm (λ max of ATOR) and 267 nm (λ max of PIO) were selected for the formation of simultaneous equation. Different aliquots were taken from stock solutions and diluted with the same solvent to prepare a series of concentrations. The calibration curves were found to be linear in concentration range of 5-50 µg/ml for ATOR and PIO respectively. The absorbance of ATOR and PIO were measured at 247nm and 267nm which are respective Wavelength of both the drugs respectively. The concentration of two drugs in mixture can be calculated by using simultaneous equation.

Method II: Q-absorbance method

Q-absorbance method uses the ratio of Absorbances at two selected wavelength,one at isoabsorptive point and other being the λmax of one of the two components. The standard solution of strength 100mcg/mL of ATOR and PIO were prepared in methanol separately. From overlain spectra of ATOR (10 µg/ml) and PIO (10 µg/ml) two wavelengths 233nm (Isoabsorbtive point) and 267 nm (λ max of PIO) were selected for the formation of Q-absorbance equation. Different aliquots were taken from stock solutions and diluted with the same solvent to prepare a series of concentrations. The calibration curves were found to be linear in concentration range of 5-50 µg/ml for ATOR and PIO respectively. The absorbance of ATOR and PIO were measured at 233nm and 267nm.

Method III: HPLC

Method Validation

(a) **Linearity.-**Calibration graph was constructed by plotting peak area vs. Concentration of ATOR and PIO and the regression equations were calculated. The calibration graph were plotted over 5 different concentrations (10, 20,30,40,50 µg/ml) using methanol. Aliquots (20µL) of each solution were injected under the chromatographic conditions.

(b) **Accuracy**- The accuracy of the methods was determined by calculating recoveries of ATOR and PIO by standard addition method. Known amounts of mixed standard solution of ATOR and PIO (80,100 and 120%) were added to prequantitated sample solutions of tablet dosage form. The amount of ATOR and PIO were estimated by applying values of peak area to the regression equation of the calibration graph.

(c) **Method precision (repeatability) -** The precision of the instrument was checked by repeatedly injecting (n=6) mixed standard of ATOR and PIO (20µg/ml).

(d) **Intermediate precision (reproducibility) -** The intraday and interday precision of proposed methods were determined by analyzing mixed standard solution of ATOR and PIO at 3 different concentrations, and three times on the same day and on three different days. The results are reported in the terms of Relative standard deviation.(RSD)

(e) **Limit of detection (LOD) and limit of quantitation LOQ**: The LOD with signal-to-noise ratio of 3:1 and with S/N ratio of 10:1 were calculated for both the drugs using the following equations

$$LOD=3.3*\sigma/s \qquad LOQ=10*\sigma/s$$

RESULTS AND DISCUSSION:

The proposed method was found to be simple, accurate, economical, and rapid for routine analysis of ATOR and PIO in combined dosage form. The accuracy of method was determined by calculating the mean percentage recovery. Precision was determined as inter and intraday variation for both the drugs. Both methods were successfully used to estimate the amount of ATOR and PIO present in marketed formulation. For the RP-HPLC method, chromatographic conditions were optimized to achieve the best resolution and peak shape for ATOR and PIO. Different mobile phases containing methanol, Acetonitrile and 0.05 M Potassium Di hydrogen phosphate were examined (data not shown), and the mobile phase methanol, acetonitrile–0.05 M Potassium di hydrogen phosphate, pH adjusted to 2.5 ±0.1 (60 :20:20, v/v/v) was selected as optimal for obtaining Well-defined and resolved peaks. The optimum wavelength for detection and quantitation was 233 nm, at which the best detector response for both the substances was obtained Straight line calibration curves were obtained for ATOR and PIO in the spectrophotometric and RP-HPLC methods. The proposed methods were also evaluated in the assay of commercially available tablets containing ATOR and PIO. Six replicate determinations were performed on the accurately weighed amounts of tablets. For ATOR, recovery (mean, %, ± SD, n = 6) was found to be 99.48 ± 1.10, 100.05±0.613 and 100.16 ± 0.616 for Methods I,II and III, respectively. For PIO, recovery was found to be 99.89 ± 0.15, 100.10±0.56 and 99.30 ± 0.35 for Methods I, II and III, respectively. For ATOR, the recovery study results ranged from 99.34 to 100.1%, 99.98 %to 100.02% and 100.1 %to 100.94 %for Methods I, II and III, respectively. For PIO, the recovery results ranged from 99.92 %to 99.75%, 99.27% to 99.23% and 100.30% to 100.33% for Methods I, II and III,

CONCLUSION:

The results confirmed that the method is simple, precise and accurate. The method can be used for the routine simultaneous analysis of ATOR and PIO in pharmaceutical preparations.

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