Research Article

SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND GLIMEPIRIDE IN TABLET DOSAGE FORM

Sadia Afrin[1], Tasnuva Haque[2], Md. Mesbah Uddin Talukder[1] and S. M. Ashraful Islam[1*]
[1]Department of Pharmacy, University of Asia Pacific, Dhanmondi, Dhaka-1209, Bangladesh.
[2]Department of Pharmacy, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka-1217, Bangladesh.
***Corresponding author's E-mail:** ashraf@uap-bd.edu

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ABSTRACT

A simple, precise, accurate and reproducible spectrophotometric method has been developed and validated for the quantification of atorvastatin and glimepiride in tablet dosage form by simultaneous equation method. Two spectrophotometers (Shimadzu, UV-1700 & UV-1601) were used for validation and absorbances were recorded at 241 nm & 231 nm as analytical wavelength for simultaneous estimation. Both the drugs followed Beer's law in concentration range of 8-22mcg/ml. The method was validated in terms of linearity, accuracy (% Recovery), precision (inter day, intraday and reproducibility) and robustness. Linearity of the method was within range (R^2 = 0.999 for both the drugs) and the % recovery was 99.04% for atorvastatin & 100.94% for glimepiride from the binary mixture. The method was found precise (% RSD< 2%) and robust. Commercial products were analyzed by the proposed method and potency was found within limit. Therefore the proposed method can be used for the simultaneous determination of atorvastatin and glimepiride from combined pharmaceutical dosage form.

Keywords: Atorvastatin calcium, Glimepiride, Method validation, UV, Quantitative analysis.

INTRODUCTION

Atorvastatin (ATV), a synthetic lipid-lowering agent, is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMGCoA) reductase which catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis[1]. Atorvastatin is currently used as calcium salt for the treatment of hypercholesterolemia[2]. Chemically it is known as [R-(R*,R*)]-2-(4- fluorophenyl)-*b*,*d*-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl] -1*H*pyrrole-1-heptanoic acid hemi-calcium salt (Figure 1).

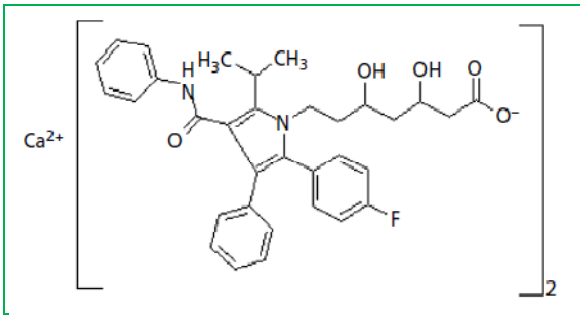

Figure 1: Structure of atorvastatin calcium.

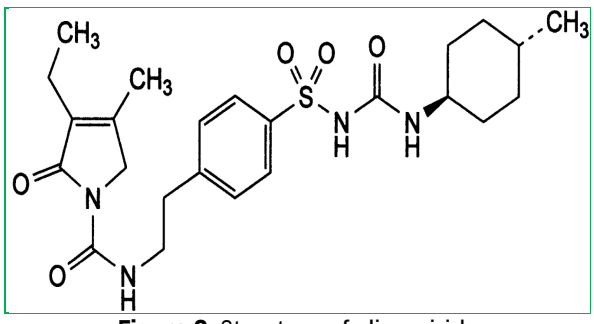

Figure 2: Structure of glimepiride

Glimepiride (GLM) is an oral blood-glucose-lowering drug which is a derivative of sulfonylurea[3]. Chemically it is known as 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl)urea (Figure 2). Glimepiride is widely used as antidiabetic drug in patient with type-2 diabetes (non-insulin-dependent diabetes).

Combination of atorvastatin and glimepiride is helpful for the treatment of hypercholesterolemia along with diabetic as atorvastatin is used for the treatment of hypercholesterolemia and glimepiride is used as hypoglycemic agent for type-2 diabetic patient. It has also been observed that patient with high level of cholesterol may develop diabetic problem later. So combination preparation containing these two drugs in a single dosage form will be available soon. Currently no method is yet reported for simultaneous estimation of these two drugs from combined pharmaceutical dosage form. Therefore, it is highly required to develop and validate an analytical method for the simultaneous estimation of atorvastatin and glimepiride in combine dosage form.

Literature survey revealed that UV, HPTLC and HPLC methods are available for atorvastatin analysis either in single formulation or in combination with another drugs[4-6]. HPLC analysis of atorvastatin along with two drugs (ramipril and aspirin) has also been reported[7]. Similarly, a survey of the analytical literature for glimepiride revealed that methods based on UV Spectrophotometry and HPLC are available for determination of glimepiride in pharmaceuticals either single or combine with other drugs[8-11].

To the best of our knowledge none of the reported analytical procedures describes a simple and satisfactory

UV spectrophotometric method for the simultaneous determination of ATV and GLM in their combined dosage forms. So the present work was undertaken to develop and validate an economic and rapid spectrophotometric method for the simultaneous determination of ATV and GLM in combination drug products that allows the analysis of a large number of samples in a short period of time.

MATERIALS AND METHODS

Instruments

A double-beam Shimadzu (Kyoto, Japan) UV-Visible spectrophotometer, Model UV-1700 PC, equipped with 1 cm quartz cells, with a fixed slit width (1 nm), wavelength accuracy of +0.5 nm (with automatic wavelength correction) was used. The drug analyses data were acquired and processed using UV Probe software (Version 2.0, Shimadzu, Japan) running under Windows XP on a Pentium PC. For scanning, the wavelength range selected was from 400 nm to 200 nm with medium scanning speed.

Reagents and Chemicals

Methanol and sodium hydroxide were analytical reagent grade and purchased from E. Merck, Darmstadt, Germany. Water was deionised and double distilled.

Working Standards

Working standard of atorvastatin calcium and glimepiride were collected from Eskayef Bangladesh Ltd as gift samples. Marketed formulation of atorvastatin tablet 10 mg and glimepiride tablet 4 mg were purchased from local drug store in Dhaka city after checking their manufacturing license number, batch number, production and expiry date.

METHOD

Preparation of standard solution

Stock solution of atorvastatin (100 mcg/ml) and glimepiride (100 mcg/ml) were prepared by dissolving 10 mg drug in 100 ml 0.1N sodium hydroxide separately. Several aliquots of standard solutions of atorvastatin (100 mcg/ml) and glimepiride (100 mcg/ml) were diluted to get standard solution across the range of 2-22 mcg/ml. Solution containing mixture of atorvastatin and glimepiride (10, 12, 15, and 18 mcg/ml atorvastatin along with 10 mcg/ml glimepiride and *vise versa*) were also prepared by diluting standard solutions.

Preparation of sample solution

Average weight of atorvastatin tablet and glimepiride tablet were calculated. Then the tablets were grinded separately to fine powder with the help of mortar and pestle. Powder containing 5 mg atorvastatin and 2 mg glimepiride was dissolved in 0.1 N sodium hydroxide, shaken for about 10 minutes and filtered through filter paper. The filtered solution was further diluted to make the final concentration of working sample equivalent to 100% of target concentration.

Method development

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for spectroscopic determination of atorvastatin and glimepiride in tablet dosage form. Simultaneous equation method was used to determine atorvastatin and glimepiride. Solutions containing 10 mcg /ml of atorvastatin and 10 mcg/ml of glimepiride were scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption for both the drugs. Atorvastatin and glimepiride showed absorbance maxima at 241 nm and 231nm respectively. Spectra for both the drugs are shown in Figure 3 to 4.

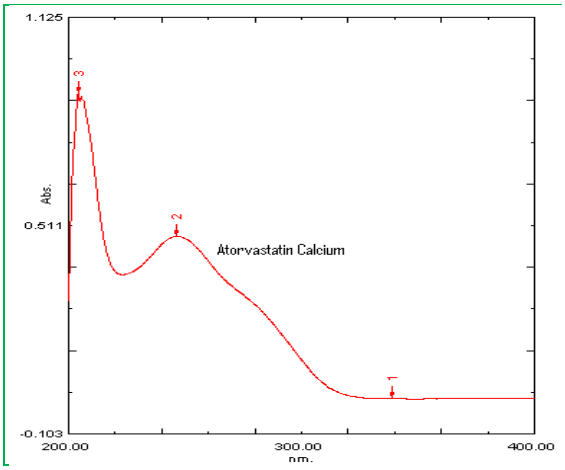

Figure 3: Spectrum of ATV

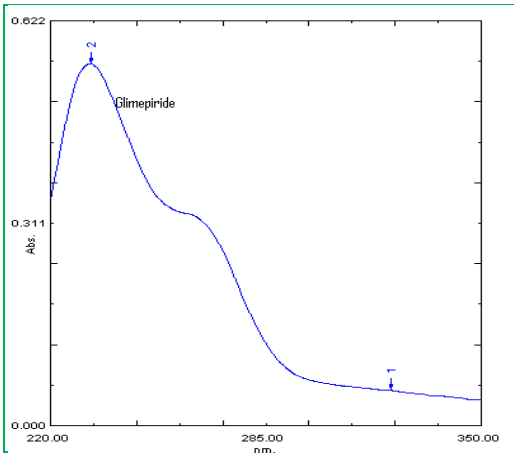

Figure 4: Spectrum of GLM

The following equations (1 and 2) were used for all the concentration calculation.

C_{ATV} = A2 ay1 – A1 ay_2 / ax_2ay_1-ax_1 ay_2 .....(1)

C_{GLM}= A1 ax2 –A2 ax_1 / ax_2 ay_1-ax_1 ay_2 .....(2)

where, A1 and A2 are absorbance of sample solution at λmax of ATV(241nm) and λmax of GLM(231nm) respectively; ax_1 and ax_2 are the absorptivities of ATV at 241nm and 231 nm respectively and ay_1 and ay_2 are the absorptivities of GLM at the two wavelengths respectively.

The absorbance of various standard solutions was recorded at the selected wavelengths and the absorptivity values were determined for atorvastatin and glimepiride. Absorptivity values for atorvastatin at 241 nm and 231 nm were 350 (ax_1) and 270 (ax_2) while respective values for glimepiride were 360 (ay_1) and 490 (ay_2).

From the absorptivity values of atorvastatin and glimepiride the simultaneous equations were derived for determination of atorvastatin and glimepiride in mixed standard solution and in its pharmaceutical formulation.

Method validation

The proposed method was validated for the parameters like linearity, accuracy, precision and robustness as per ICH guidelines[12].

Linearity

The linearity of an analytical method is its ability to elicit that test results are proportional to the concentration of drug in samples within a given range. Linearity of the method was determined by constructing calibration curves. Standard solutions of atorvastatin and glimepiride of different concentrations level (8-22 mcg/ml) were used for this purpose. Each measurement was carried out in six replicates and the absorbances were plotted against the concentrations to obtain the calibration curves and correlation coefficients. Characteristic parameters for regression equation ($y = a + bx$) of the method were obtained by least squares treatment of the results and these parameters were used to confirm the good linearity of the method.

Accuracy

Accuracy indicates the deviation between the mean value found and the true value. The accuracy is the closeness of agreement between the true value and test result. Accuracy was determined by means of recovery experiments. Solution containing known concentration of atorvastatin and glimepiride was used for this purpose. From the absorbance at two selected wave length potency was calculated. The accuracy was assessed from the test results as the percentage of the drug recovered by the assay.

Precision

The precision of the method was investigated with respect to repeatability (inter assay precision), intermediate precision (inter day precision) and reproducibility (inter laboratory trial). Repeatability was determined by performing three repeated analysis of the four standard solutions (10, 12, 15, 18 µg/ml atorvastatin along with 10 mcg/ml glimepiride and *vise versa*) of standard mixture solution on the same day, under the same experimental conditions. Experiment was done from 9.00 am to 9.00 pm. % RSD was calculated to determine the reparability. Intermediate precision of the method was assessed by carrying out the analysis of standard solutions on three different days (inter-day) in the same laboratory. For reproducibility the procedure repeated in another lab by using equipment (Shimadzu spectrophotometer model UV-1601). The relative standard deviation (% RSD) was calculated in order to assess the precision of the method.

Robustness

To determine the robustness different solvent composition was used. Percent recovery was calculated for both the drug. Analytical methods are generally known as robust if percent recovery is within 98-102%

Analysis of market products

The proposed method was used to determine the potency of commercially available tablets (atorvastatin tablet 10 mg and glimepiride tablet 4 mg). Six replicate determinations (n=6) were carried out.

RESULTS AND DISCUSSION

The proposed method was found to be simple with linearity in the concentration range (8-22mcg/ml). Correlation coefficient was 0.999 for atorvastatin and glimepiride which proves the high linearity of the method. (Figure 5 and 6)

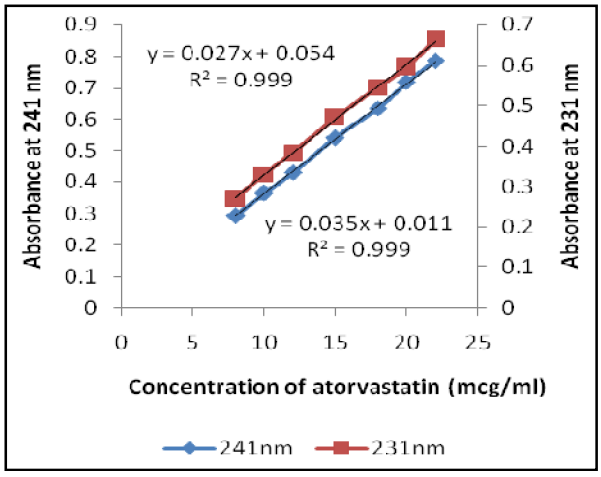

Figure 5: Calibration curve of atorvastatin at 241 nm (◊) and 231 nm (□)

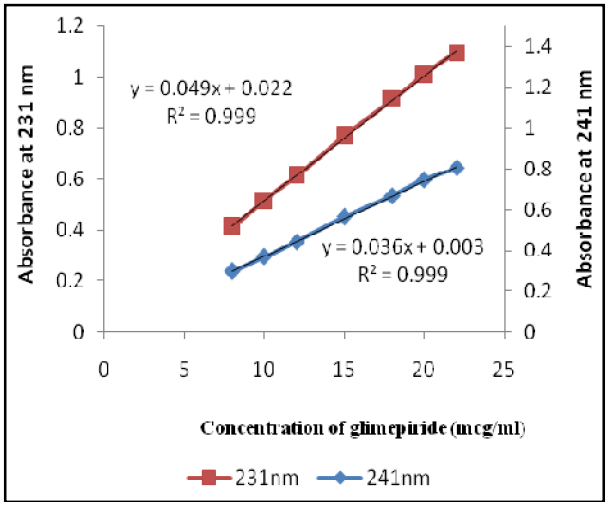

Figure 6: Calibration curve of glimepiride at 241 nm (◊) and 231 nm (□)

The method was found to be accurate as indicated by results of recovery studies as %RSD was not more than 2% (table 1).

Results of precision (repeatability, intermediate precision and reproducibility) are summarized in table 2. Percent recovery was found 98.75% ± 0.321 to 99.667% ± 0.158 for atorvastatin and 99.13% ± 0.133 to 100.8% ± 0.532 for glimepiride with % RSD value less than 1%. All the results indicate that the method is highly precise.

The method is found robust as no significant effect was observed in the recovery of drugs. % recovery was 98% to 102% (table 3).

Market products analysis results are summarized in Table 4. Potency of both the drugs was within limit.

Table 1: Data showing accuracy of the developed method

Drug	Drug conc. (mcg/ml)	% Recovery (n=6)	SD	% RSD
Atorvastatin	10	101.30	0.529	0.522
	15	102.10	0.382	0.374
	18	101.10	0.371	0.367
Glimepiride	10	100.67	0.441	0.438
	15	100.42	0.443	0.441
	18	101.17	0.485	0.479

Table 2: Data showing precision of the developed method

Parameters		Atorvastatin	Glimepiride
Repeatability	% Recovery	99.417	99.533
	SD	0.234	0.674
	% RSD	0.235	0.677
Intermediate precision	% Recovery	99.667	99.333
	SD	0.158	0.384
	% RSD	0.159	0.387
Reproducibility	% Recovery (UV 1700)	99.250	100.800
	SD	0.432	0.532
	% RSD	0.435	0.528
	% Recovery (UV 1601)	98.750	99.133
	SD	0.321	0.833
	% RSD	0.325	0.840

Table 3: Results of robustness

Solvent Composition	Drug in standard solution (mcg/ml)		Measured conc.(mcg/ml)		% Recovery	
	ATV	GLM	ATV	GLM	ATV	GLM
0.1 N NaOH	10	12	10.11	12.09	101.10	100.75
	10	12	10.09	11.94	100.90	99.50
	10	12	9.95	12.11	99.50	100.92
0.1 N NaOH with 2% Methanol	10	12	10.01	12.12	100.10	101.00
	10	12	98.92	12.05	989.20	100.42
	10	12	99.31	11.84	993.10	98.67

Table 4: Results of analysis of tablet dosage forms containing ATV and GLM

Drug	Atorvastatin	Glimepiride
Label claim (mg) (n=6)	10.00	4.00
Observed amount (mg) (n=6)	10.02	3.98
Potency (%)	100.20	99.50
SD	0.92	1.62
% RSD	0.92	1.63

CONCLUSION

From this validation study we can conclude that the developed UV method is accurate, rapid, precise, reproducible and inexpensive with acceptable correlation co-efficient, RSD (%) and standard deviation. The method is versatile and valuable for simultaneous determination of atorvastatin and glimepiride in bulk or pharmaceutical dosage form (individual or combine). Simplicity of sample preparation and use of low cost reagents are the additional benefit of this method. So this method can be used in the quality control department for potency and dissolution study.

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