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Research Article

Simultaneous Estimation and Validation of Multicomponent Formulation in Tablets by Spectrophotometric Method

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ABSTRACT

Two methods for the simultaneous estimation of atorvastatin calcium, glimepiride and metformin hydrochloride in three-component solid dosage forms have been developed. First method involved solving simultaneous equations based on measurement of absorbance at three wavelengths, 247.2 nm, 224.8 nm and 236 nm as the λ_{max} of atorvastatin calcium, glimepiride and metformin hydrochloride respectively while in the second method the instrument is preprogrammed to collect and compile the spectral data from the scan of standards and produces the result by matrix calculations. The linearity for three drugs was in the range of 5-30 $\mu\text{g/ml}$. The procedures were successfully applied for the simultaneous determination of three drugs in laboratory prepared mixtures and commercial tablet preparation. High recovery and low % COV revealed the reliability of the methods indicating proposed methods are accurate and precise for the simultaneous estimation of three drugs in pure and tablet formulation.

Keywords: Atorvastatin calcium, Glimepiride, Metformin hydrochloride, Simultaneous equation method. Multiwavelength Spectroscopy

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1. INTRODUCTION

Chemically, atorvastatin calcium is [R-(R*,R*)-2-(4-fluorophenyl)- β -dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptonic acid-calcium salt (2:1) trihydrate used as a synthetic cholesterol-lowering agent. Glimepiride is a sulphonylurea antidiabetic drug and is chemically 3-ethyl-2,5-dihydro-4-methyl-N-[2-[4-[[[(trans-ethyl cyclohexyl)amino]carbonyl]amino]sulphonyl]ethyl]2-oxo-1H-pyrrole-1-carboxamide Metformin hydrochloride is an oral antidiabetic drug and is chemically N, N-dimethyl imidodicarbonimidic diamide Analytical methods, such as RP- HPLC [1], and HPTLC [2] have been reported for the estimation of atorvastatin calcium from its formulations. UHPLC-MS/MS method for determination of atorvastatin calcium in human plasma is also reported [3]. A literature study found that HPLC [4], LC-MS/MS [5] techniques are recorded for the assessment of glimepiride. RP HPLC [6] and stability indicating HPTLC [7] method for estimation of glimepiride and metformin hydrochloride is reported. UV Spectrophotometric [8] method for estimation of metformin alone is reported. Simultaneous estimation of metformin, glimepiride and atorvastatin in combined tablet dosage form by UPLC [9] and Stability Indicating RP-HPLC are also

reported [10]. A combination of atorvastatin calcium, glimepiride, and metformin hydrochloride is commercially available in a tablet dosage form. Literature shows that there is no technique of analyzing these three drugs in combination at the same time by spectrophotometry. So we communicate here rapid and cost-effective quality-control tool for routine quantitative analysis of all three drugs in their combined dosage forms by spectrophotometry.

2. EXPERIMENTAL

2.1. Materials

UV-visible double beam spectrophotometer, Shimadzu model 1700 with 1 nm spectral bandwidth, ± 0.3 nm wavelength precision and a couple of 10 mm matching quartz cells were used. The commercially available tablet, cdpro 2 (Label claim: atorvastatin calcium 10 mg, glimepiride 2 mg and metformin hydrochloride 500 mg) was procured from the local market.

2.2. Selection of common solvent

Methanol was selected as a common solvent for developing spectral characteristics of the drug. The selection was made

after assessing the solubility of three drugs in different solvents.

2.3. Preparation of standard stock and calibration curve

The standard stock solutions of atorvastatin calcium, glimepiride, and metformin hydrochloride were prepared by dissolving 10 mg of each drug in 10mL of methanol in 10 mL of the volumetric flask to get a solution containing 1000 µg/mL of each drug. From the above solution, 0.1 ml solution was taken and diluted to 10 ml with methanol to get a solution containing 10 µg/ mL.

Working standard solutions of 10 µg/mL were scanned in the entire UV range of 400-200 nm to determine the λ_{max} of these drugs. The λ_{max} of atorvastatin calcium, glimepiride, and metformin hydrochloride was found to be 247.2 nm, 224.8 nm, and 236 nm respectively (Figure-1). Six working standard solutions for three drugs having concentration 5, 10, 15, 20, 25, 30 µg/mL was prepared in methanol from the stock solution. The absorbance of resulting solutions for three drugs were measured at their respective λ_{max} and a calibration curve was plotted against concentration to get the linearity and regression equation of three drugs.

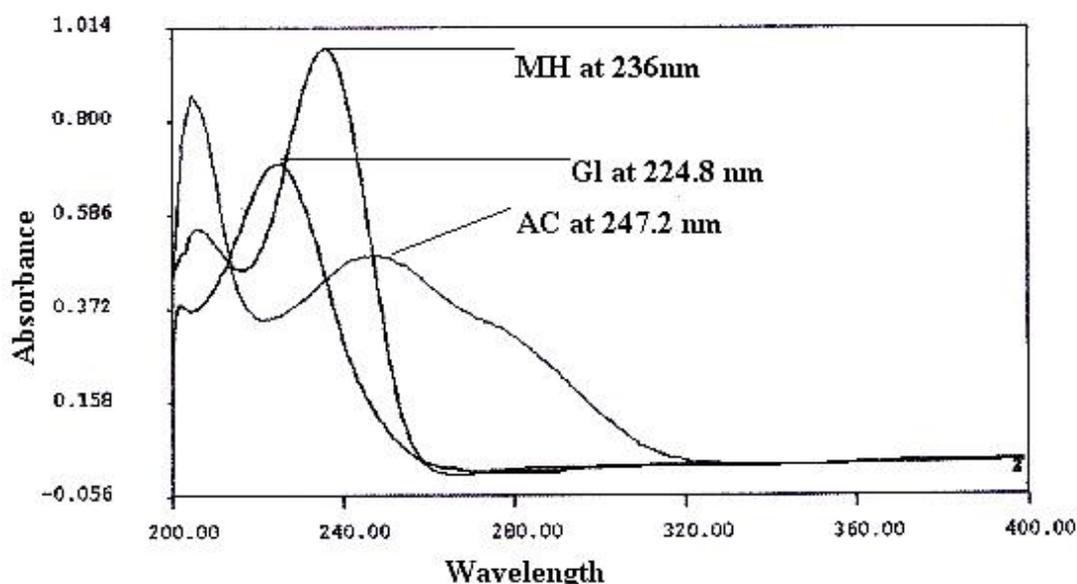


Figure 1. Overlain spectra of atorvastatin calcium, glimepiride and metformin hydrochloride

2.4 Method I: Simultaneous equation method

Simultaneous equation method of analysis was based on the absorption of drugs (atorvastatin calcium, glimepiride, and metformin hydrochloride) at the wavelength maximum of each other. Three wavelengths selected for the development of the simultaneous equations were 247.2 nm, 224.8 nm, and 236 nm λ max of atorvastatin calcium, glimepiride, and metformin hydrochloride respectively. The absorbance of

three drugs was measured at 247.2 nm, 224.8 nm and 236 nm. The absorptivity values determined at 247.2 nm, 224.8 nm and 236 nm for atorvastatin were 0.0457 (ax1), 0.0342 (ax2), 0.0418 (ax3); for glimepiride 0.0142 (ay1), 0.0633 (ay2), 0.0409 (ay3); for metformin hydrochloride 0.0438 (az1), 0.0621 (az2), 0.0931 (az3). These values are means of six estimations. Thus absorptivity coefficients were substituted in the following equations to obtain the concentration of three drugs.

$$A_1 = 0.0457x C_{AC} + 0.0142x C_{GI} + 0.0438x C_{MH} \dots\dots\dots \text{Eqn.1}$$

$$A_2 = 0.0342x C_{AC} + 0.0633x C_{GI} + 0.0621x C_{MH} \dots\dots\dots \text{Eqn. 2}$$

$$A_3 = 0.0418x C_{AC} + 0.0409x C_{GI} + 0.0931x C_{MH} \dots\dots\dots \text{Eqn.3}$$

Where C_{AC}, C_{GI}, and C_{MH} are concentration the of atorvastatin, glimepiride and metformin hydrochloride respectively. A₁, A₂, and A₃ are the absorbance of the mixture at 247.2 nm, 224.8 nm, and 236 nm respectively and concentration of three drugs in the sample were determined using Eqn.1, 2 and 3.

2.6. Method II: Multiwavelength Spectroscopy

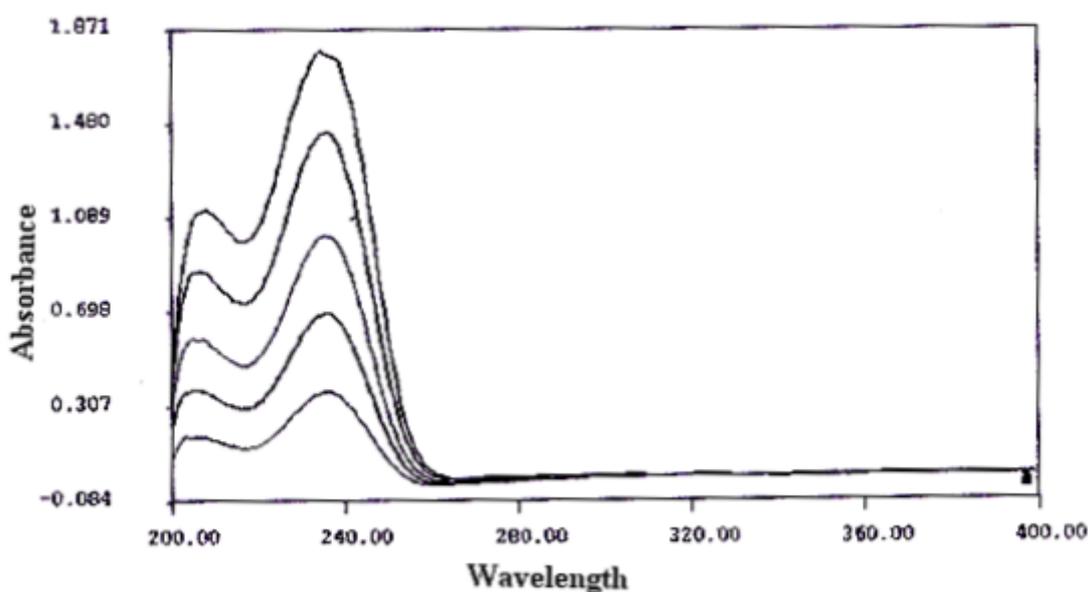
In this method, the instrument is preprogrammed to collect and compile the spectral data from the scan of standards and produces the result by matrix calculations. Five mixed standards of atorvastatin calcium, glimepiride and metformin hydrochloride having concentrations in µg/mL 0.1:0.02:5, 0.2:0.04:10, 0.3:0.06:15, 0.4:0.08:20 and

0.5:0.1:25 were prepared in methanol by diluting appropriate volumes of standard stock solutions and scanned between 400 nm to 200 nm. Sampling wavelengths (247.2 nm, 224.8 nm, 236 nm, and 240nm) were selected on the trial and error basis. The concentration of individual drugs was feed to the multi-component mode of the instrument. The instrument collects and compiles the spectral data from mixed standards and the concentration of each component was obtained by spectral data of sample solution regarding that of five mixed standards. As outlined in Method I, a tablet sample solution was prepared. The spectrophotometric analysis of the resulting solution was carried out using the multicomponent mode of the instrument.

Table 1. Analysis data of tablet formulation

Method	Drug	Label claim mg/tab	Amount found* mg/tab	Label claim (%)	S.D.*	% COV	S.E.*
I	AC	10	9.980	99.80	0.9894	0.9913	0.4039
	GI	2	2.0034	100.17	0.3771	0.3764	0.1539
	MH	500	500.65	100.13	0.7527	0.7517	0.3072
II	AC	10	9.985	99.85	0.5471	0.5479	0.2233
	GI	2	2.011	100.56	0.6521	0.6484	0.2662
	MH	500	502.1	100.43	0.4781	0.4760	0.1951

AC: Atorvastatin Calcium, GI: Glimepiride, MH: Metformin hydrochloride, S.D.: Standard deviation, COV: Coefficient of variation, S.E.: Standard error, *Average of six estimation of tablet formulation

**Figure 2.** Overlain Spectra of Mixed Standard of Atorvastatin Calcium, Glimepiride and Metformin Hydrochloride

2.5. Analysis of the tablet formulations

Twenty tablets of marketed formulation were accurately weighed and powdered. A standard addition method has been used for drug assessment. A quantity of powder equivalent to 5 mg of metformin hydrochloride was weighed and dissolved in 10 mL of methanol. Then the solution was filtered through Whatman filter paper no 41. From the above solution, 0.2 mL of solution was taken and diluted to 10 mL with the same solvent to get a solution containing 10 µg/mL of metformin hydrochloride and the corresponding concentration of atorvastatin calcium and glimepiride. To this, 0.05 mL of stock solution (1000 µg/mL) of pure atorvastatin calcium and glimepiride was added and volume was made up to the mark with methanol. The purpose of this addition was to bring the concentration of atorvastatin calcium and glimepiride in the linearity range. With this addition, the concentration of atorvastatin calcium, glimepiride and metformin hydrochloride in the samples was brought to 5.2, 5.04 and 10 µg/mL respectively. Analysis

operation with tablet formulation was repeated six times. The result of the analysis of tablet formulation was reported in Table 1.

2.7. Validation 11

2.7.1 Accuracy

The accuracy of the established technique was verified by conducting 80%, 100%, 120% replicate analysis (n= 3) retrieval research following ICH standards at three distinct concentration concentrations. Standard medication solutions were introduced here to a pre-analyzed sample solution and then the proportion of drug content was calculated. Table 2 disclosed the results of precision research. From the recovery study, it was clear that the method is very accurate for quantitative estimation of atorvastatin calcium, glimepiride and metformin hydrochloride in a tablet dosage form as the statistical results were within the acceptance range (S.D. < 2.0).

Table 2. Result of recovery studies

Tablet Brand	Method	Recovery level (Added amount)	Percent recovery+ SD#		
			AC	GI	MH
cdpro 2 (Label claim: atorvastatin calcium 10 mg, glimepiride 2 mg and metformin hydrochloride 500 mg)	I	80%	100.20 +0.342	100.45+0.457	99.50+0.453
		100%	99.50+ 0.571	100.1+0.742	100.54+0.462
		120%	98.50+0.457	100.84+0.561	99.47+0.471
	II	80%	98.56+0.354	98.40+0.483	101.45+0.542
		100%	100.40+0.451	98.50+0.561	100.20+0.562
		120%	98.50+0.587	101.60+0.874	99.30+0.541

AC: Atorvastatin Calcium, GI: Glimepiride, MH: Metformin hydrochloride, S.D.: Standard deviation,

Average of three estimation at each level of recovery

2.7.2. Precision

By studying repeatability and intermediate precision, precision was determined.

2.7.2.1. Repeatability

The result of repeatability indicates the accuracy over a short time and inter-assay precision under the same operating conditions. The standard deviation, coefficient of variance and standard error were calculated for three drugs. Repeatability with tablet formulation was conducted six times. The statistical assessment findings are presented in Table 1.

2.7.2.2. Intermediate Precision (Inter-day and Intra-day precision)

Intermediate precision was carried out by doing intra and inter-day precision study. In intraday study concentration of three drugs was calculated on the same day at an interval of one hour. In the inter-day study, the concentration of drug content was calculated on three different days. Study expresses within laboratory variation on different days. In both intra- and inter-day precision study for methods percentage of COV was not more than 1.0 % indicates good intermediate accuracy (Table 3).

Table 3. Optical characteristics data and validation parameters

Parameters	Values		
	AC	GI	MH
Maximum absorbance (λ max)	247.2nm	224.8nm	236nm
Beer's law limit ($\mu\text{g/ml}$)	5-30	5-30	5-30
Absorptivity*	0.0457	0.0633	0.0931
Correlation coefficient*	0.997	0.983	0.986
Intercept*	0.048	0.106	0.059
Slope*	0.042	0.056	0.088
LOD* ($\mu\text{g/ml}$)	0.1278	0.4532	0.0241
LOQ*($\mu\text{g/ml}$)	1.6587	0.7354	0.5781
Intra-Day* (Precision) (% COV)	0.4581	0.3562	0.7845
Inter-Day (Precision) (% COV) n=3	0.8650	0.5874	0.6521

AC: Atorvastatin Calcium, GI: Glimepiride, MH: Metformin hydrochloride, S.D.: Standard deviation, COV: Coefficient of variation, * Average of six determination.

2.7.3. Linearity

Appropriate dilutions of standard stock solutions have been tested according to the techniques established for each drug. The Beer- Lambert's concentration range was found to be 5-30 $\mu\text{g/mL}$ for atorvastatin calcium, glimepiride, and metformin hydrochloride. The linearity data for both methods are presented in Table 3.

2.7.4. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD & LOQ of atorvastatin calcium, glimepiride, and metformin hydrochloride by proposed methods were determined using calibration standards. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$ respectively, where S is the slope of the calibration curve and σ is the standard deviation of response.

3. RESULTS AND DISCUSSION

The Beer- Lambert's concentration range was found to be 5-30 µg/mL for atorvastatin calcium, glimepiride, and metformin hydrochloride at 247.2 nm, 224.8 nm and 236 nm wavelengths and coefficient of correlation were found 0.997, 0.983 and 0.986 respectively. All three drugs showed good regression values at their respective wavelengths and the results of the recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed methods.

Percentage estimation of three drugs found in tablet dosage form were 99.80, 100.17 and 100.13 as per method I while 99.85, 100.56, 100.43 as per method II for atorvastatin calcium, glimepiride, and metformin hydrochloride respectively with standard deviation <2 (Table 1).

In method II five mixed standard and three sampling wavelengths were selected through rational experimentation keeping in view the number of drugs in the formulation and molar absorptivity coefficients of all three drugs (Fig. 2). The method requires no manual calculations, produces comparable results to the first method and is more suitable as compared to the method I.

The validity and reliability of the proposed methods were assessed by recovery studies. Sample recoveries for both the methods are in good agreement with their respective label claims, which suggested non-interference of formulation additives in estimation (Table-2).

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, coefficient of variance and standard error were calculated for atorvastatin calcium, glimepiride, and metformin hydrochloride (Table 1). The intermediate precision study expresses within laboratory variation on different days. In both intra and inter-day precision studies for both the methods %COV were not more than 1.0 % indicates good intermediate precision (Table 3).

The LOD values were 0.1278, 0.4532 and 0.0241 µg/mL and LOQ values were 1.6587, 0.7354 and 0.5781 µg/mL for atorvastatin calcium, glimepiride and metformin hydrochloride respectively. Low values of LOD and LOQ indicated good sensitivity of proposed methods.

4. CONCLUSION

The results of the analysis of two drugs from tablet formulation using all the three developed methods were found close to 100% for atorvastatin calcium, glimepiride, and metformin hydrochloride, the standard deviation was satisfactorily low indicating accuracy and reproducibility of the methods. Recovery trials have been satisfactory, showing that excipients are not interfering. The developed methods were found to be simple, rapid, and accurate and can be used

for routine estimation of three drugs from tablet formulations.

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