



SIMULTANEOUS ESTIMATION OF SIMVASTATIN AND METFORMIN HYDROCHLORIDE IN BULK AND SOLID DOSAGE FORMS

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ABSTRACT

A simple, accurate, precise, sensitive and a highly selective ultra violet spectrophotometric method has been developed for the simultaneous estimation of simvastatin and metformin hydrochloride in bulk and solid dosage form. The estimation of simvastatin was carried out at 247 nm while metformin hydrochloride was estimated at 232.2 nm. The developed method was validated for linearity, range, precision, recovery studies and interference study for mixture. All these parameters showed the adaptability of the method for the quality control analysis of the drug in bulk and in combination formulations.

Keywords: Simvastatin, Metformin Hydrochloride, UV Spectrophotometric, Dosage forms, Type 2 diabetes.

INTRODUCTION

Chemically simvastatin is Butanoic acid, 2,2-dimethyl-1,2,3,7,8,8a-hexahydro-3,7 dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)-ethyl]-1-naphthalenyl ester, [1S-[1 α ,3 α ,7 β ,8 β (2S*,4S*),8a β]] as shown in Figure-1¹. Therapeutically simvastatin is a lipid lowering agent and commercially produced via a multistage fermentation process originating from cultures of a strain of *Aspergillus terreus*². After oral administration, the inactive lactone (simvastatin) is hydrolyzed to the corresponding β -hydroxyacid form³. This is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyses the conversion of HMG-CoA to mevalonate (rate limiting step in the biosynthesis of cholesterol)⁴.

Metformin hydrochloride (*N,N*-dimethylimidodicarbonimidic diamide hydrochloride) (fig. 2)⁵ is an oral antihyperglycemic drug used in the management of type 2 diabetes⁶. When administered orally, it decreases the hepatic glucose production, intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake along with its utilization⁷⁻⁸.

The combination of both the drugs would be beneficial for the treatment of diabetes and hyperlipidemia. However the combination therapy of two drugs if they are administered in the form of a single formulation, a simultaneous estimation would be required. Literature survey revealed that no UV spectrophotometric method has been reported yet for the simultaneous estimation of these two drugs. So it is worthwhile to pursue the present work.

EXPERIMENTAL

Instrumentation

A Systronic UV-Visible Spectrophotometer 2203, with 1 cm matched quartz cell was used for the absorbance measurement over the range of 220-280 nm.

Reagents and Chemicals

Samples of simvastatin and metformin hydrochloride used in this study were gifted by GMH laboratories India and Abhilasha Pharma Pvt. Ltd., India respectively. All the reagents and chemicals used were of analytical grade.

Determination of wavelength and calibration graph

Standard stock solution was prepared by dissolving simvastatin in methanol to make final concentration of 1000 µg/ml. Different aliquots were taken from stock solution and diluted with pH 6.8 phosphate buffer to prepare the series of concentration from 5-15 µg/ml. The same procedure was used for metformin hydrochloride vice versa but drug was dissolved in water and dilutions from stock solution were prepared from 2-10 µg/ml using pH 6.8 phosphate buffer. The absorption λ_{\max} for both the drugs were measured and the calibration curves were prepared by plotting absorbance versus concentration of the drugs.

Effect of solvent on wavelength of both the drugs

The effect of solvent on λ_{\max} of both the drugs was carried out by dissolving metformin hydrochloride and simvastatin in 100 ml mixture of methanol and water (1:1).

Validation of analytical method

The absorbance of sample solutions of metformin hydrochloride and simvastatin were measured at 232.2 nm and 247 nm respectively. The results were calculated by the following formula using Vierodt's method⁹⁻¹⁰

$$A_1 = ax_1 Cx + ax_2 Cy \quad \text{at 232.2 nm}$$

$$A_2 = ay_1 Cx + ay_2 Cy \quad \text{at 247 nm}$$

Where, A_1 and A_2 are absorbance of diluted mixture of drugs at 232.2 nm and 247 nm respectively, Cx and Cy are the concentration of metformin hydrochloride and simvastatin respectively (µg/ml), ax_1 and ax_2 are absorptivities of metformin hydrochloride at 232.2 nm and 247 nm respectively, ay_1 and ay_2 are absorptivities of simvastatin at 232.2 nm and 247 nm respectively.

Linearity and range

The linearity of an analytical procedure is its ability to obtain test results and directly proportional to the concentration of analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample. It has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity¹¹.

Accuracy and Precision

Accuracy and precision (repeatability and intermediate precision) were investigated by analyzing three concentrations of simvastatin and metformin hydrochloride mixtures in three independent replicates on the same day (Intra-day accuracy and precision) and on three consecutive days (Inter-day accuracy and precision). Intra-day and Inter-day relative standard deviation were calculated¹¹.

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were evaluated from the calibration curves plotted in concentration range of 5-15 µg/mL for simvastatin and 2-10 µg/mL for metformin hydrochloride, with formula $LOD = 3.3 S.D/S$ and $LOQ = 10 S.D/S$ (where S.D = Standard Deviation and S= slope of the calibration curve). The LOD and LOQ for each drug were thus obtained¹¹.

Recovery studies

To study the accuracy of the proposed method, recovery studies were carried out by the standard addition technique of drug with excipients. A known amount of metformin hydrochloride and simvastatin was added to the preanalyzed sample solution of drug with excipients in the three different concentrations. Percentage recoveries of three concentrations were calculated¹¹.

RESULTS AND DISCUSSION

Statistical evaluation of analysis was carried out. The data obtained from the proposed method showed suitability of method. The values of relative standard deviation were satisfactorily low (≤ 2).

Determination of wavelength and calibration graph

The λ_{\max} of simvastatin was found to be 247 nm in pH 6.8 phosphate buffer (fig.4). The absorbance was measured at 247nm against pH 6.8 phosphate buffer as a blank. The calibration curve was prepared by plotting absorbance versus concentration of drug (fig. 5).

The λ_{\max} of metformin hydrochloride was found to be 232.2 nm in pH 6.8 phosphate buffer (fig.3).The absorbance was measured at 232.2 nm against pH 6.8 phosphate buffer as a blank and the calibration curve was prepared (fig. 6).

Effect of solvent on wavelength of both the drugs

No shift in λ_{\max} of both the drugs was observed (Fig. 7 and 8)

Results for validation of analytical method

The method was validated with respect to linearity and range, accuracy and precision, limit of detection and limit of quantitation, and recovery studies.

Linearity and Range

The prepared aliquots of simvastatin (5-15 $\mu\text{g/ml}$) were scanned for absorbance at 247 nm. The absorbance range was found to be 0.140-0.440. These aliquots obeyed Beer-Lambert's law with correlation coefficient (R^2) of 0.9982 and for metformin hydrochloride the aliquots prepared (2-16 $\mu\text{g/ml}$) were scanned at 232.2 nm. Absorbance range was found to be 0.181-1.353 with correlation coefficient of 0.9977 (Fig 6). The optical characteristics are shown in Table 1.

Accuracy and precision

The low RSD values obtained for repeatability and intermediate precision indicated good precision of the method. The data evaluated has been summarized in Table 2 and Table 3.

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) calculated for simvastatin was found to be 0.333 and 1.009 respectively and for metformin hydrochloride 0.201 and 0.609 respectively.

Recovery Studies

The percentage recoveries of three concentrations (5, 10 and 15 $\mu\text{g/ml}$) were found within the limit, indicative of high accuracy. The high percent recoveries indicate no interference from ingredients and excipients that might be present in formulation. Data of recovery studies are summarized in Table 4 and Table 5.

CONCLUSION

The developed spectrophotometric method was validated for simultaneous estimation of simvastatin and metformin hydrochloride using linearity and range, accuracy and precision and recovery studies. The relative standard deviations for all parameters were found to be less than two, indicated the validity of method. The assay results obtained by this method were fair agreement. So the developed method can be used for routine quantitative simultaneous estimation of simvastatin and metformin hydrochloride in various dosage forms.

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Table-1: Optical characteristics for Simvastatin and Metformin Hydrochloride

Sr. No.	Parameters	Simvastatin	Metformin Hydrochloride
1	Wavelength (λ_{\max})	247nm	232.2nm
2	Beer's law limit ($\mu\text{g/ml}$)	5-15	2-16
3	Correlation coefficient (R^2)	0.9982	0.9977
4	Slope	0.0286	0.0842

Table-2: Results of Repeatability of Metformin Hydrochloride and Simvastatin

Sr. No.	Conc. of metformin HCl taken ($\mu\text{g/ml}$)	Conc. of metformin HCl observed ($\mu\text{g/ml}$) \pm S.D.	% Recovery of metformin HCl	Conc. of simvastatin taken ($\mu\text{g/ml}$)	Conc. Of simvastatin observed ($\mu\text{g/ml}$) \pm S.D.	% Recovery of simvastatin
1	5	4.83 \pm 0.0015	96.6	5	5.11 \pm 0.0026	102.18
2	10	9.81 \pm 0.0051	98.1	10	9.72 \pm 0.0028	97.2
3	15	14.67 \pm 0.0557	97.8	15	14.83 \pm 0.0227	98.866

Each value is average of three determinations
R.S.D of metformin hydrochloride = 0.213
R.S.D of simvastatin = 0.0952

Table-3: Results of Intermediate Precision of Metformin Hydrochloride and Simvastatin

Sr. No.	Drug	Conc. of drug taken ($\mu\text{g/ml}$)	Average conc. found in Intra days studies ($\mu\text{g/ml}$) \pm S.D.	Average conc. found in Inter days studies ($\mu\text{g/ml}$) \pm S.D.
1	Metformin HCl	10	9.58 \pm 0.1061	9.65 \pm 0.1550
2	Simvastatin	10	10.47 \pm 0.0212	10.39 \pm 0.1479

Each value is average of three determinations
R.S.D. of metformin hydrochloride = 1.1075 (Intra days) and 1.6062 (Inter days)
R.S.D. of simvastatin = 0.20261 (Intra days) and 1.4244 (Inter days)

Table-4: Results of recovery studies for Metformin Hydrochloride

Sr. No.	Excipients added	Concentration of metformin hydrochloride taken (µg/ml)	Concentration of simvastatin added (µg/ml)	Concentration observed (µg/ml) ± S.D.
1	HPMC(50cps) 20 mg	10	5	10.49 ± 0.0919
2	Lactose 100 mg	10	10	9.81 ± 0.0051
3	MCC 100 mg	10	15	9.78 ± 0.0076

Each value is average of three determinations
R.S.D = 0.3474

Table-5: Results of recovery studies for Simvastatin

Sr. No.	Excipients added	Conc. of simvastatin taken (µg/ml)	Conc. of metformin HCl added (µg/ml)	Conc. observed (µg/ml) ± S.D.
1	HPMC (50cps) 20 mg	10	5	9.59 ± 0.003
2	Lactose 100 mg	10	10	9.72 ± 0.0029
3	MCC 100 mg	10	15	10.05 ± 0.0017

Each value is average of three determinations
R.S.D = 0.026

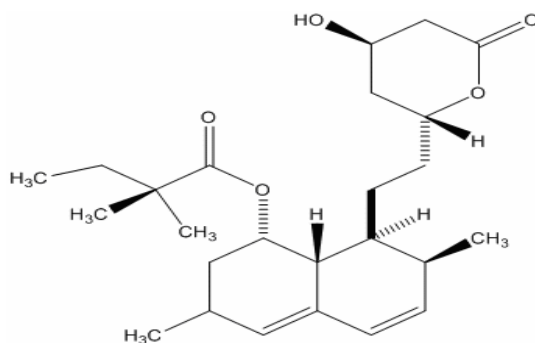


Fig.-1:Chemical structure of simvastatin

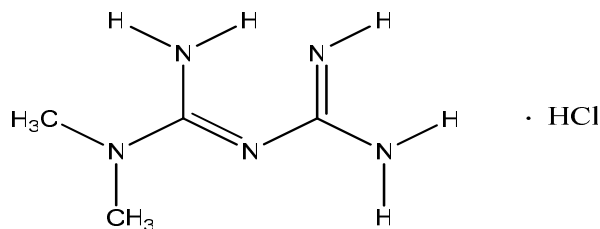


Fig.-2:Chemical structure of metformin hydrochloride

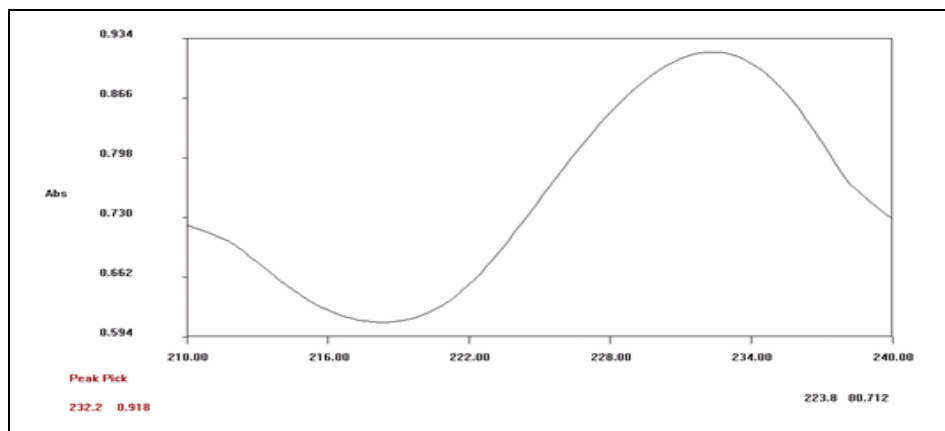


Fig.-3: UV Absorption Spectra of Metformin Hydrochloride

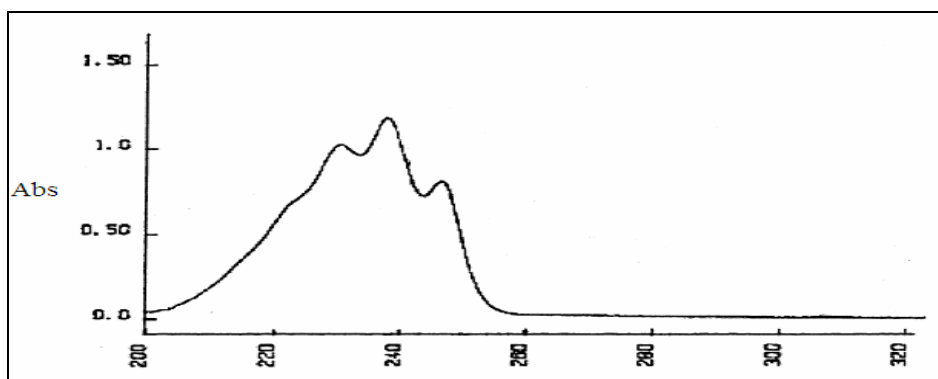


Fig.-4: UV Absorption Spectra of Simvastatin

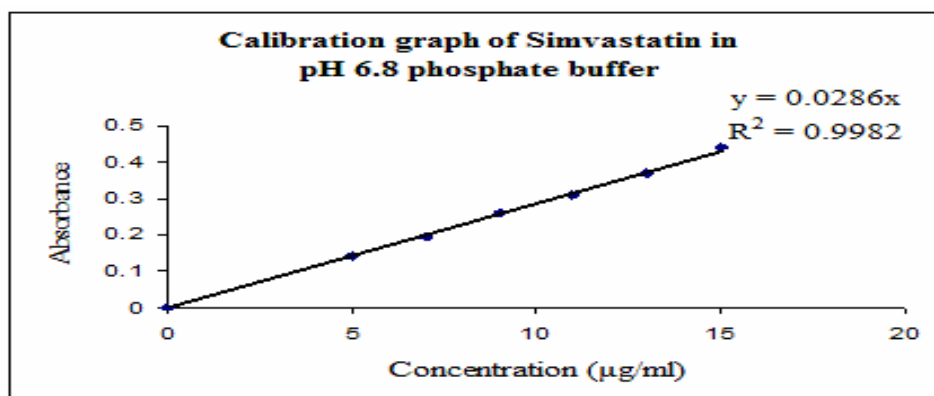


Fig.-5: Calibration graph of Simvastatin at 247 nm

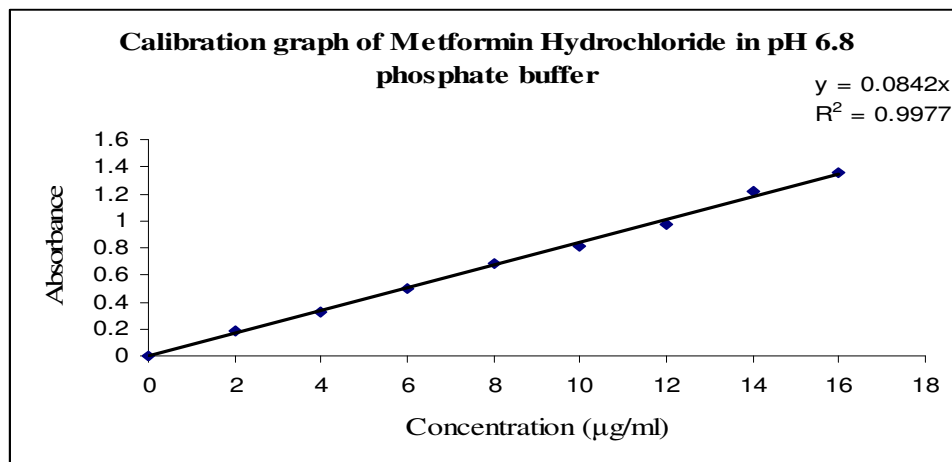


Fig.-6: Calibration graph of Metformin Hydrochloride at 232.2 nm

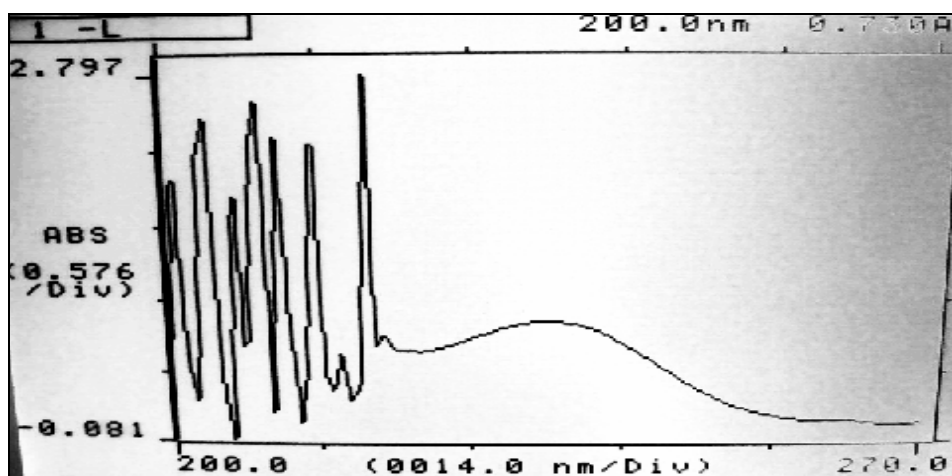


Fig.-7: Effect of solvent on λ_{max} of Metformin Hydrochloride

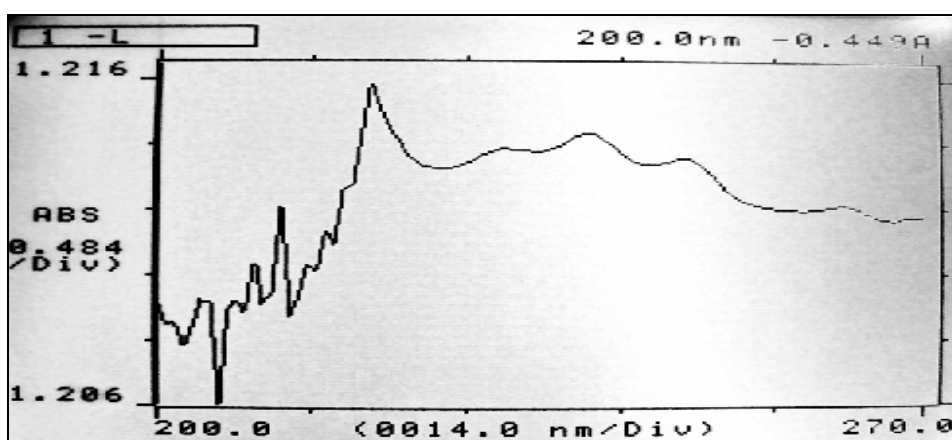


Fig.- 8: Effect of solvent on λ_{max} of Simvastatin

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