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Research Article

Simultaneous UV Spectrophotometric Methods for Estimation of Metformin HCl and Glimepiride in Bulk and Tablet Dosage Form

Audumbar Digambar Mali*[1], Seeta Mali[2], Ashpak Tamboli[2], Ritesh Bathe[1]

[1]*Department of Pharmaceutics, Sahyadri College of Pharmacy, Methwade, Sangola-413307, Solapur, Maharashtra, India*
[2]*Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade, Sangola-413307, Solapur, Maharashtra, India*

***Correspondence Info:**
Audumbar Digambar Mali
Department of Pharmaceutics,
Sahyadri College of Pharmacy,
Methwade, Sangola- 413307,
Solapur, Maharashtra, India
Email: maliaudu442@gmail.com

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Abstract

Simple, precise, economical, fast and reliable two UV methods have been developed for the simultaneous estimation of Metformin HCl and Glimepiride in bulk and pharmaceutical dosage form. Method A is Absorbance maxima method, which is based on measurement of absorption at maximum wavelength of 236 nm and 228 nm for Metformin HCl and Glimepiride respectively. Method B is area under curve (AUC), in the wavelength range of 217-247 nm for Metformin HCl and 213-239 nm for Glimepiride. Linearity for detector response was observed in the concentration range of 5- 25µg/ml for Metformin HCl and 5-25 µg/ml for Glimepiride. The accuracy of the methods was assessed by recovery studies and was found to be 100.23 % and 99.67 % for Metformin HCl and Glimepiride respectively. The developed method was validated with respect to linearity, accuracy (recovery), precision and specificity. The results were validated statistically as per ICH Q2 R1 guideline and were found to be satisfactory. The proposed methods were successfully applied for the determination of for Metformin HCl and Glimepiride in commercial pharmaceutical dosage form.

1. Introduction

Metformin HCl (MET) chemically N; N dimethylimidodicarbonimidic diamide hydrochloride is used as antidiabitic drug from the biguanide class used in the management of type 2 diabetes. Major action of metformin lay in increasing glucose transport across the cell membrane in skeletal muscle. [1-4]

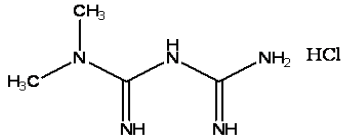

Figure 1: Chemical structure of Metformin HCl

Glimepiride is 1-{(p-[2-(3-ethyl-4-methyl- 2-oxo-3- pyrroline-1-carboxamide) ethyl] phenyl) sulfonyl}-3- (trans-4-methylcyclohexyl) urea, is 3rd generation sulfonylurea derivative used for the treatment of type II diabetes mellitus. [5-7]

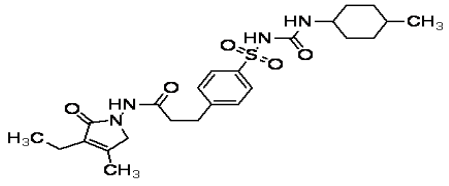

Figure 2: Chemical structure of Glimepiride

A survey of pertinent literature revealed that in estimation of individual as well as combination of Metformin HCl and Glimepiride. Simultaneous determinations of Metformin HCl and Glimepiride dosage form were also reported like HPLC [8, 9], RP-HPLC [10-13], LC [14] and UV-Spectroscopy [15-19]. Therefore an attempt was made to develop a new rapid and sensitive UV Spectrophotometric method and to validate as per ICH-guidelines. A comprehensive literature research reveals the lack of a Spectrophotometric analytical method for simultaneous estimation of Metformin HCl and Glimepiride in pharmaceutical formulations. A successful attempt was made to develop accurate, precise and simple method of analysis for estimation of both the drugs in combined dosage form.

2. Materials and Methods

2.1 Apparatus and instrumentation

A shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spectra lab UCB 40, India).Calibrated volumetric glassware (Borosil®) was used for the validation study.

2.2 Materials

Reference standard of Metformin HCl and Glimepiride API were supplied as gift sample by Lupin Laboratory Park Aurangabd. The commercial formulation Gluconorm-G 4 with label claim 500 mg Metformin HCl and 4 mg Glimepiride per tablet were purchased from local market Mangalwedha, Dist:-Solapur.

2.3 Method development

2.3.1 Preparation of standard stock solution

Stock solution was prepared by diluting 10 mg of each drug in sufficient quantity of methanol in separate volumetric flask and volume was made up to 100 ml to get the concentrations of 100 µg/ml for each drug. Dilutions from stock solution were prepared in the range of 5-25 µg/ml for Metformin HCl and 5-25 µg/ml for Glimepiride. Methanol was used as a blank solution.

2.3.2 Method A: Absorption Maxima Method

For the selection of analytical wavelength, standard solution of Metformin HCl and Glimepiride were scanned in the spectrum mode from 400 nm to 200 nm separately. From the spectra of drug λmax of Metformin HCl, 236 nm [Fig.3], and λmax of Glimepiride, 228 nm [Fig.4], were selected for the analysis. Aliquots of standard stock solution were made and calibration curve was plotted. [20-22]

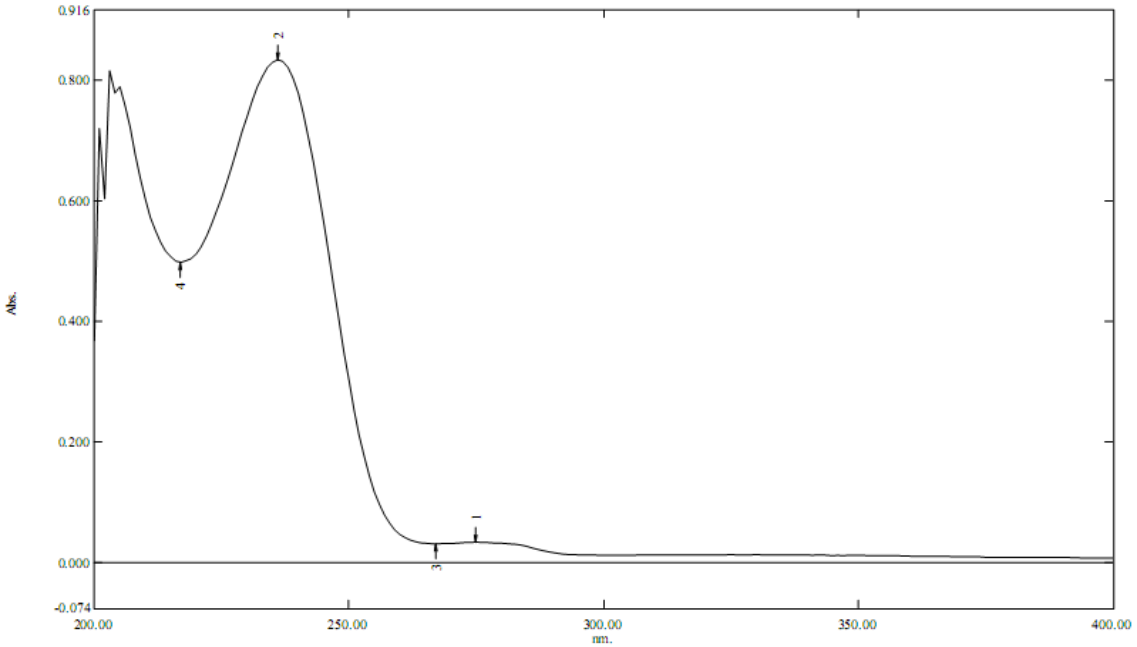

Figure 3: It shows λmax of Metformin HCl

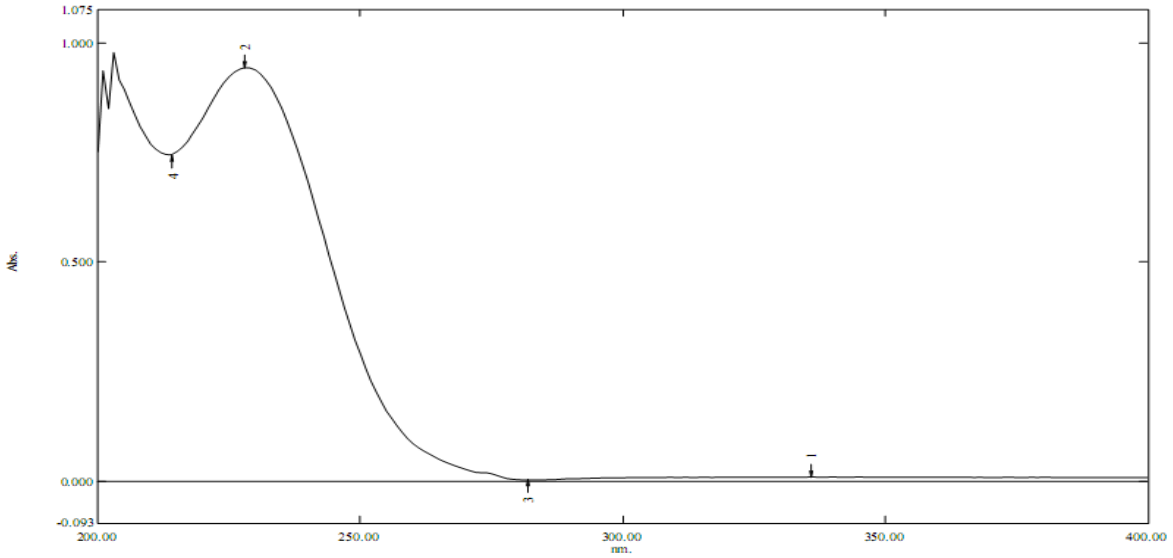

Figure 4: It shows λmax of Glimepiride

2.3.3 Simultaneous estimation of Metformin HCl and Glimepiride:

The wavelength maxima of Metformin HCl and Glimepiride were determined and found to be 236 nm (λ1) and 228 nm (λ2) respectively where there was no interference among the drugs. The overlain spectrum is shown in Fig. 5.

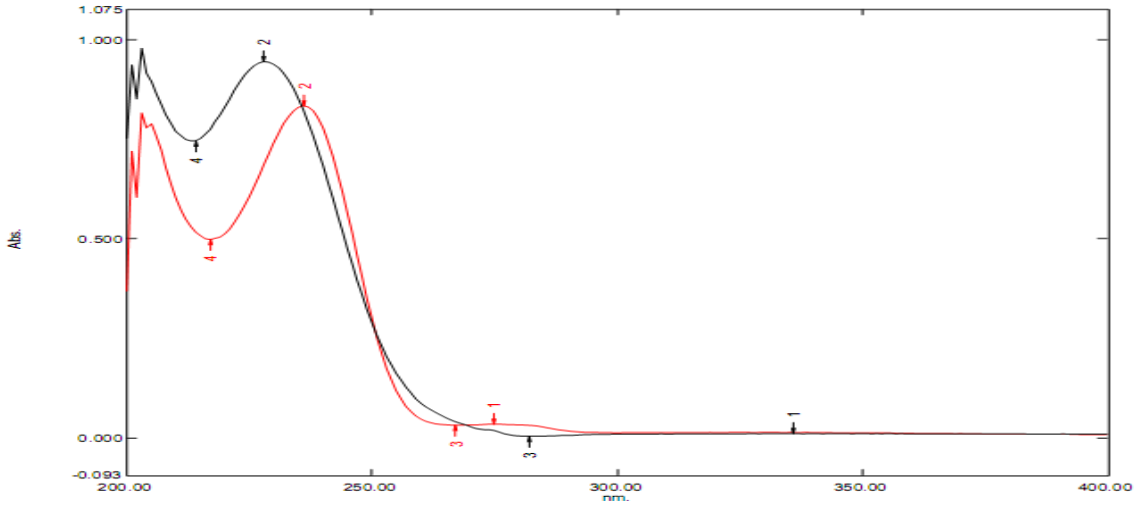

Figure 5: Isobestic point of Metformin HCl and Glimepiride

2.3.4 Method B: Area under Curve Method

From the spectra of drug obtained after scanning of standard solution of Metformin HCl and Glimepiride separately, area under the curve in the range of 217-247 nm and 213-239 nm was selected for the analysis. The calibration curve was prepared in the concentration range of 5-25 µg/ml for Metformin HCl and 5-25 µg/ml for Glimepiride at their respective AUC range. Both drugs followed the Beer-Lambert's law in the above mentioned concentration range. The calibration curves were plotted as absorbance against concentration of HCl and Glimepiride. The coefficient of correlation (r), slope and intercept values of this method are given in Table 2.

Area calculation: $(\alpha+\beta) = \int_{\lambda 2}^{\lambda 1} A d\lambda$

Where, α is area of portion bounded by curve data and a straight line connecting the start and end point, β is the area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis λ1 and λ2 are wavelength range start and end point of curve region. [23-25]

2.3.5 Application of the proposed methods for the determination of Metformin HCl and Glimepiride in tablet dosage form

For the estimation of drugs in the tablet formulation, 20 tablets were weighed and weight equivalent to 500 mg of Metformin HCl and 4 mg of Glimepiride was transferred to 50 ml volumetric flask and ultrasonicated for 20 minutes and volume was made up to the mark with methanol. The solution was then filtered through a Whatmann filter paper (No.42). The filtrate was appropriately diluted further.

Table 1: Table shows Results of Analysis of Tablet Formulation

Method	Drug	Label Claim mg	Sample Solution Concentration (µg/ml)	Amount found (%)*±	% Recovery	%RSD
A	Metformin HCl	500 mg	20	100.18 ±1.24	100.69	0.6394
B	Metformin HCl	500 mg	20	99.47±0.98	99.07	
A	Glimepiride	4 mg	20	101.29±1.47	101.54	0.6481
B	Glimepiride	4 mg	20	99.69±1.76	101.96	

*n=3, % RSD = % Relative Standard Deviation.

In Method-A, the concentration of Metformin HCl and Glimepiride was determined by measuring the absorbance of the sample at 236 nm and 228 nm respectively in zero order spectrum modes. By using the calibration curve, the concentration of the sample solution was determined.

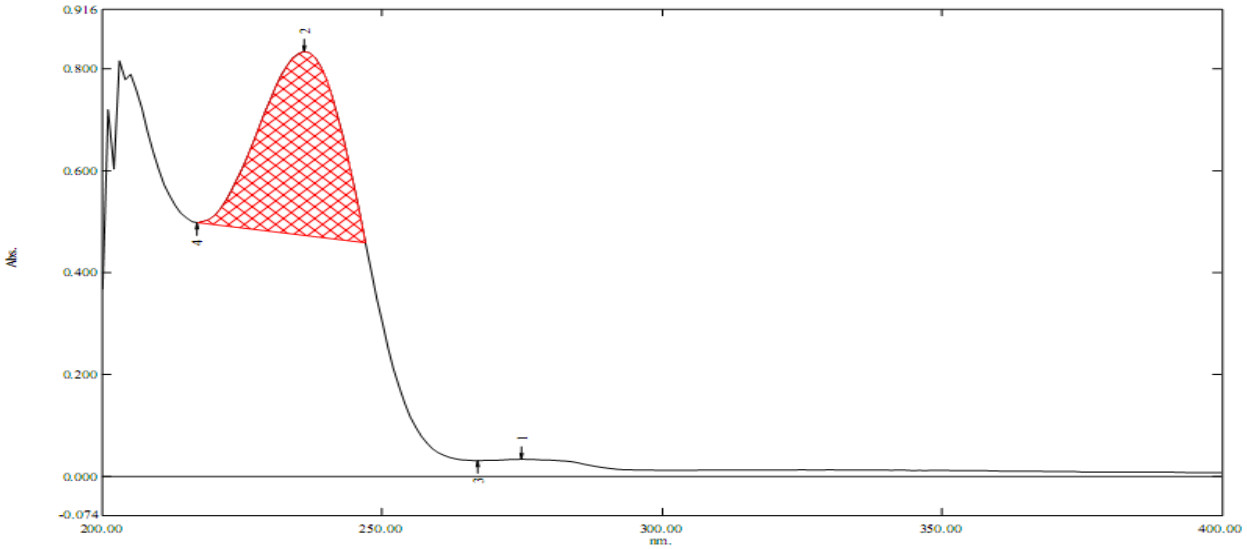

Figure 6: It shows AUC of Metformin HCl

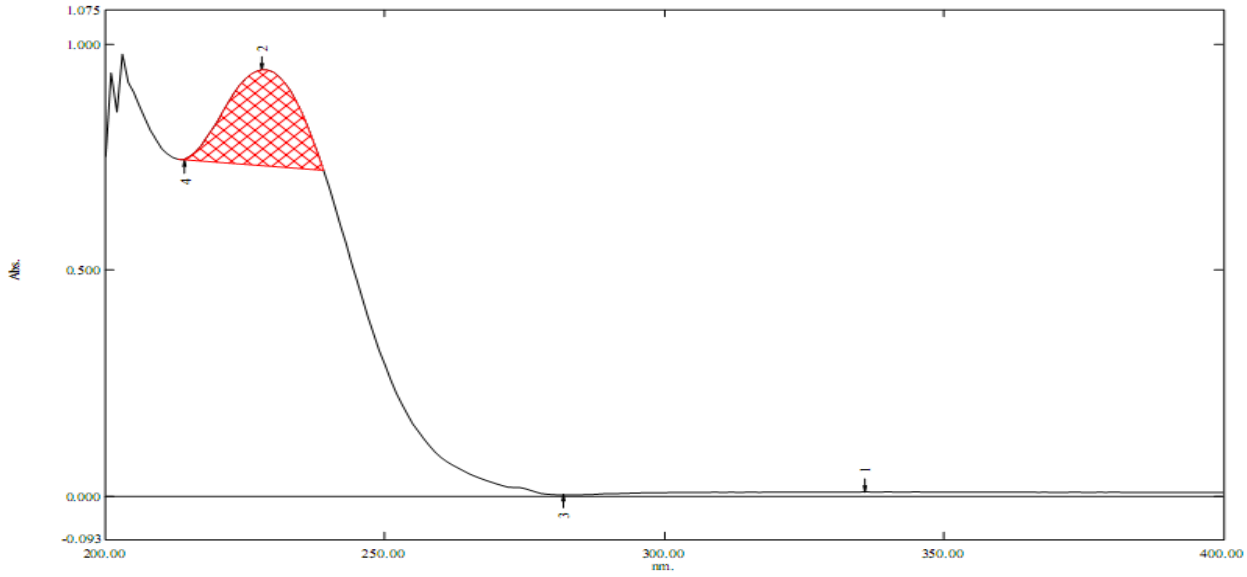

Figure 7: It shows AUC of Glimepiride

In Method-B, the concentration of Metformin HCl and Glimepiride was determined by measuring area under curve in the range of 217-247 nm and 213-239 nm. By using the calibration curve, the concentration of the sample solution was determined.

3. Validation of the Developed Methods: [26-31]

The methods were validated with respect to accuracy, linearity, precision and selectivity.

3.1 Accuracy: - Accuracy of an analysis was determined by systemic error involved. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. Recovery studies carried out for both the methods by spiking standard drug in the powdered formulations 80%, 100%, 120% amount of each dosage content as per ICH guidelines**.**

3.2 Linearity: - The linearity of measurement was evaluated by analyzing different concentration of the standard solution of Metformin HCl and Glimepiride. Result should be expressed in terms of correlation co-efficient.

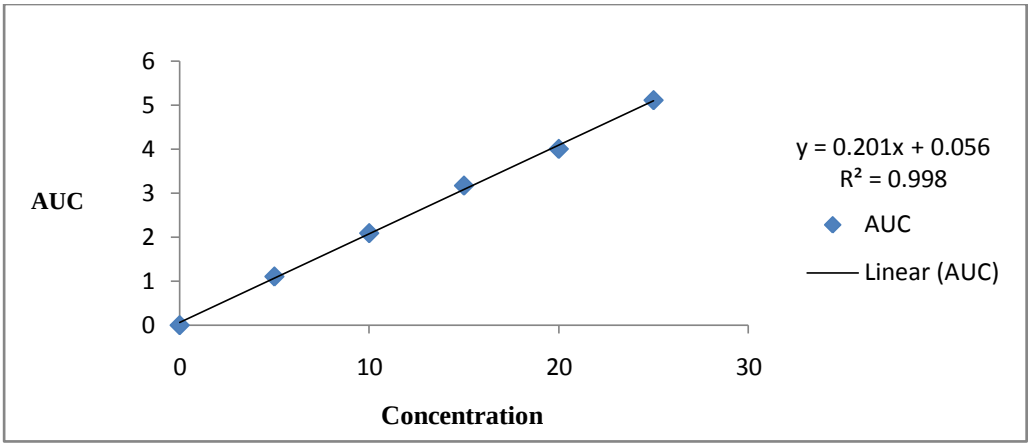

Figure 8: Calibration curve for Metformin HCl at 236 nm

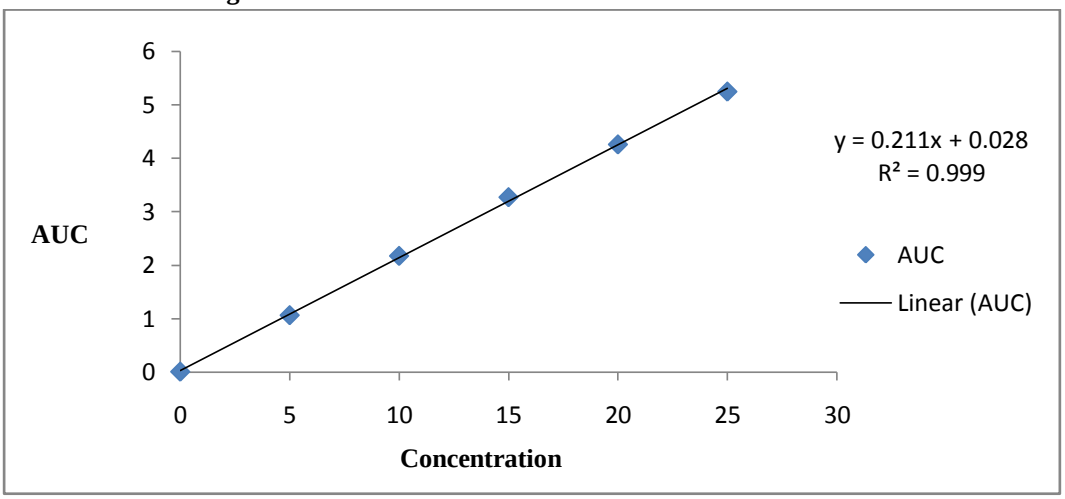

Figure 9: Calibration curve for Glimepiride at 228 nm

3.3 Precision: - The reproducibility of the proposed method was determined by performing tablet assay at different time intervals (morning, afternoon and evening) on same day (Intra-day assay precision) and on three different days (Inter-day precision). Result of intra-day and inter-day precision is expressed in % RSD.

3.4 Sensitivity: - The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations LOD = 3xσ/ S and LOQ = 10xσ/S, where σ is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be 0.7480 µg/ml and 2.4491µg/ml respectively of Metformin HCl and 0.7904 µg/ml and 2.3718 µg/ml of Glimepiride.

Table 2: Optical Characteristics and Precision

Sr. No.	Parameter	Metformin HCl	Glimepiride
1	λ range	200-400 nm	200-400nm
2	Regression Equation (y= mx+c)	Y=0.201x+0.056	Y=0.211x+0.028
3	Measured wavelength	236 nm	228 nm
4	Linearity range	5-25µg/ml	5-25µg/ml
5	Slope	0.201	0.211
6	Intercept	0.056	0.028
7	Correlation coefficient (R^2)	0.998	0.999
8	Limit of Detection (LOD) µg/ml	0.7480	0.7904
9	Limit of Quantitation (LOQ) µg/ml	2.4491	2.3718

Table 3: Results of drug content and analytical recovery of Metformin HCl and Glimepiride

Excess drug added to the analyte (%)	Drug	% Recovery		% RSD	
		Method A	Method B	Method A	Method B
80	Metformin HCl	100.23	101.58	0.5962	0.5874
100		98.28	100.92	0.4780	0.7149
120		99.10	99.69	0.4982	0.5241
80	Glimepiride	99.67	100.93	0.6589	0.4120
100		98.84	101.21	0.6317	0.5715
120		100.29	101.28	0.5470	0.6583

Table 4: Results of Intra-day and Inter-day Precision

Method	Drug	Intra-day Precision		Inter-day Precision	
		SD	%RSD	SD	%RSD
A	Metformin HCl	0.785	0.428	0.681	0.381
B		0.774	0.398	0.589	0.265
A	Glimepiride	0.248	0.845	0.187	0.107
B		0.198	0.745	0.142	0.099

4. Results and Discussion

The methods discussed in the present work provide a convenient and accurate way for analysis of Metformin HCl and Glimepiride in its bulk and pharmaceutical dosage form. Absorbance maxima of Metformin HCl at 236 nm and Glimepiride at 228 nm were selected for the analysis. Linearity for detector response was observed in the concentration range of 5-25 µg/ml for Metformin HCl and 5-25 µg/ml for Glimepiride. Percent amount found for Metformin HCl and Glimepiride in tablet analysis was found in the range of 100.18 %, 99.47 and 101.29, 99.69 % respectively [Table 1]. Standard deviation and coefficient of variance for three determinations of tablet formulation, was found to be less than ± 2.0 indicating the precision of the methods. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. % recovery for Metformin HCl and Glimepiride was found in the range of 100.23 % and 99.67 % values of standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of all the methods. % RSD for Intraday assay precision for Metformin HCl was found to be 0.785 and 0.774 for Method A and B, and for Glimepiride, 0.248 and 0.198 for Method A and B. Interday assay precision for Metformin HCl was found to be 0.681 and 0.589 for Method A and B and for Glimepiride 0.187 and 0.142 for Method A and B. The LOD and LOQ were found to be 0.7480 µg/ml and 2.4491µg/ml respectively of Metformin HCl and 0.7904 µg/ml and 2.3718 µg/ml of Glimepiride. Based on the results obtained, it is found that the proposed methods are accurate, precise, reproducible & economical and can be employed for routine quality control of Metformin HCl and Glimepiride in bulk drug and its pharmaceutical dosage form.

5. Conclusion

UV spectrophotometric methods for Metformin HCl and Glimepiride were developed separately in bulk and tablet dosage form by, Absorbance maxima method and Area under curve method. Further, UV Spectrophotometric methods for the simultaneous estimation of Metformin HCl and Glimepiride were in bulk and combined dosage form. The methods were validated as per ICH guidelines. The standard deviation and % RSD calculated for these methods are <2, indicating high degree of precision of the methods. The results of the recovery studies showed the high degree of accuracy of these methods. In conclusion, the developed methods are accurate, precise and selective and can be employed successfully for the estimation of Metformin HCl and Glimepiride in bulk and pharmaceutical dosage form.

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