



Development and Validation of Direct Spectrophotometric Method for the Estimation of Glimepiride

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Authors' contributions

This work was carried out in collaboration between both authors. Author DC designed the study, performed the statistical analysis and wrote the first draft of manuscript. Author LA did the experimental work. Both authors managed the literature searches and read and approved the final manuscript.

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ABSTRACT

Aims: To develop simple, sensitive and direct spectrophotometric methods for the estimation of widely prescribed antidiabetic hypoglycemic agent Glimepiride in pure and pharmaceutical dosage form.

Place and duration of study: Research Centre of Taywade Group of Institution Koradi, Nagpur affiliated to Rashtrasant Tukadoji Maharaj Nagpur University Nagpur between June 2020 and March 2021.

Methodology: Two spectrophotometric methods were developed based on ion-pair formation of drug with Cresol Red dye (Method A) and Bromophenol Blue dye (Method B) in methanol and chloroform. The possible reaction mechanism was proposed with evaluation of statistical parameters. The methods were validated for its application to determine Glimepiride in bulk as well as in pharmaceutical formulations

Results: The Beer's law was found linear in the range 10 - 60 μgml^{-1} at 450 nm for method A and 2 - 20 μgml^{-1} at 578 nm for method B, respectively. The linear regression equation obtained by applying least square regression analysis for Glimepiride were found to be Absorbance = 0.0136 x Concentration in μgml^{-1} + 0.028; $R^2 = 0.9965$ for method A and Absorbance = 0.0428x

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Concentration in μgml^{-1} $+0.0722$; $R^2 = 0.9949$ for method B. The Sandell's sensitivity was found to be 0.0696 and 0.0177 for method A and B respectively. The apparent molar absorptivity calculated to be for both methods were 6.6724×10^3 and $2.0999 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$.

Conclusion: Two direct spectrophotometric methods for determination of Glimepiride have been developed successfully and validated for sensitivity, accuracy, precision, repeatability and robustness as per ICH guidelines. The developed methods are suitable for the routine estimation of Glimepiride in pure and dosage form.

Keywords: Glimepiride; spectrophotometric; bromophenol blue; cresol red; ion-pair complex.

1. INTRODUCTION

Glimepiride (GMP) is chemically 1- [[p- [2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1- carboxamido) ethyl] phenyl] sulfonyl] - 3 - (trans - 4 - methyl cyclohexyl urea). [Fig. 1]. Glimepiride is a third-generation sulphonyl urea derivative in the treatment of type II diabetes mellitus. It reduces blood glucose levels by stimulating insulin secretions from the β -cells of pancreas. It also increases peripheral insulin sensitivity thereby decrease insulin resistance.

Literature survey revealed that Glimepiride was simultaneously determined with Metformin hydrochloride, Pioglitazone hydrochloride and Rosiglitazone maleate by using various analytical methods [1-4]. The estimation of Glimepiride in plasma was reported by Tandem mass Spectrophotometry [5]. The determination of Glimepiride as single dose was described by Liquid Chromatography [6], HPLC [7], RP-HPLC [8] and UV-Spectrophotometry [9-11]. The kinetic study [12] of Glimepiride and simple visible spectrophotometry based on charge transfer complex formation [13] and extractive Spectrophotometry [14] were also described.

Most of these methods require expensive instrumentation. Some of the spectrophotometric methods described are only applicable in the ultra violet range of the spectrum and need costly solvents. Reported visible spectrophotometric methods are scanty which needs extraction and are time consuming. No direct spectrophotometric method is reported in literature.

The aim of the present work to develop and validate simple, sensitive and accurate direct spectrophotometric methods by ion-pair complex formation with Cresol red and Bromophenol blue.

The developed method can be applied for quantification of Glimepiride in pure and dosage form which can be served as a quality control tool.

2. MATERIALS AND METHODS

2.1 Instrumentation

A UV-Visible Spectrophotometer (Elico SL-150 model of Elico Pvt. Ltd, India) with a spectral band width of 2 nm wavelengths and a matched quartz cell of 10 mm pathlength was employed. All weighing was performed in an analytical balance (Contech Pvt. Ltd., India).

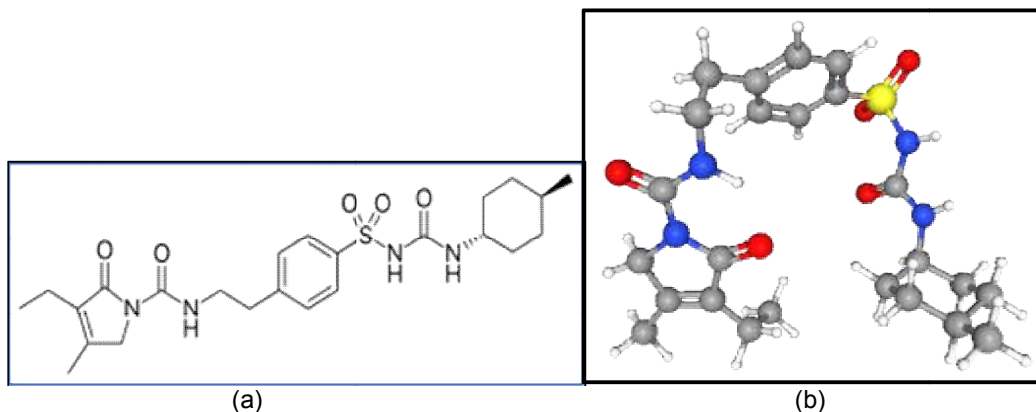


Fig.1. Structure of Glimepiride (a) Molecular structure (b) Ball and stick structure

2.2 Chemicals

All the reagent and chemicals used were of analytical grade. Pure Glimepiride was purchased from Sigma- Aldrich, India. The solvents methanol, chloroform and dye Cresol red (CSR) and Bromophenol blue (BPB) were purchased from Merck India. The tablets Amaryl (Sanofi Aventis, India), Dibiglim (Novartis, India) and Glader-1 (Lupin Pharma, India) were procured from local market.

2.3 Reagents

2.3.1 Standard stock solution of GMP (1×10^{-3} M)

An accurately weighed quantity of about 12.27 mg of Glimepiride [GMP] was transferred in a 25 ml volumetric flask and volume was made by methanol and chloroform to get a standard stock solution of 1×10^{-3} M.

2.3.2 Standard stock solution of CSR (1×10^{-3} M)

An accurately weighed quantity of about 96.9 mg of Cresol red [CSR] was transferred in a 25 ml volumetric flask and volume was made by methanol and chloroform to get a standard stock solution of 1×10^{-3} M.

2.3.3 Standard stock solution of BPB (1×10^{-3} M)

An accurately weighed amount of about 167.5 mg of Bromophenol blue [BPB] was dissolved in a 25 ml volumetric flask by methanol and

chloroform to get a solution of concentration 1×10^{-3} M.

2.3.4 Sample solution of GMP

Commercially available tablets of Glimepiride (GMP) were procured from the local market. Twenty tablets were pulverized and amount equivalent to 10 mg of the tablet was transferred to a 25 ml volumetric flask. The volume was made by methanol and chloroform. The solution was homogenized on magnetic stirrer for half an hour and filter through Whatman filter paper number 42.

3. RESULTS AND DISCUSSION

The different spectral parameters concern with spectrophotometric determination of Glimepiride by ion-pair complex formation with Cresol red and Bromophenol blue in chloroform was studied.

3.1 Determination of λ_{max}

An aliquot of 1.0 ml and 2.0 ml of standard stock solution of GMP was transferred to separate 10 ml volumetric flask. The same volume of CSR solution was added to each flask and volume was made by chloroform. The instantly formed colored chromogen was scanned in wavelength region of 400-700 nm and the maximum wavelength of absorbance was determined. Similar procedure was applied with BPB solution. The color of the complexes of GMP with CSR (Method A) and BPB (Method B) were yellow and red which shows absorbance maxima at 450 nm and 578nm, respectively [Figs. 2, 3].

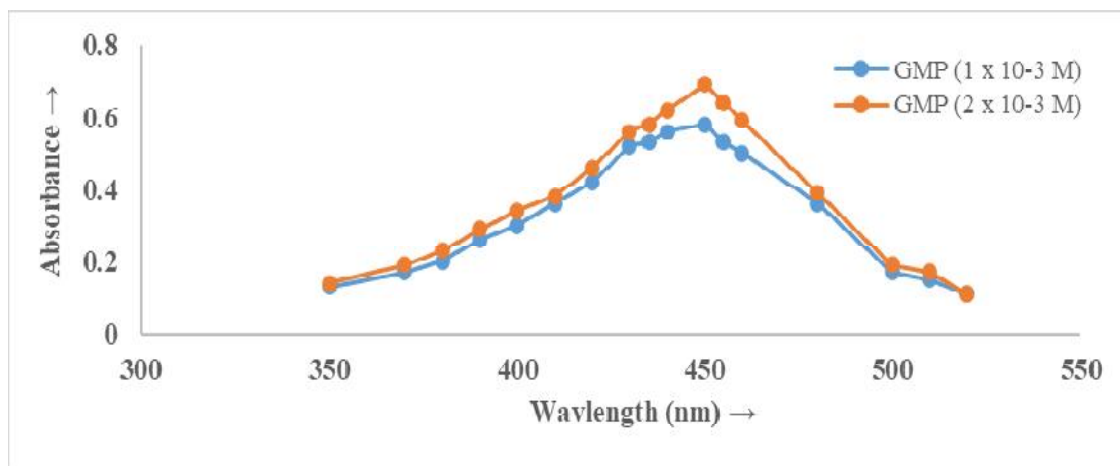


Fig. 2. Absorption spectra of the complex [GMP]: [CSR]

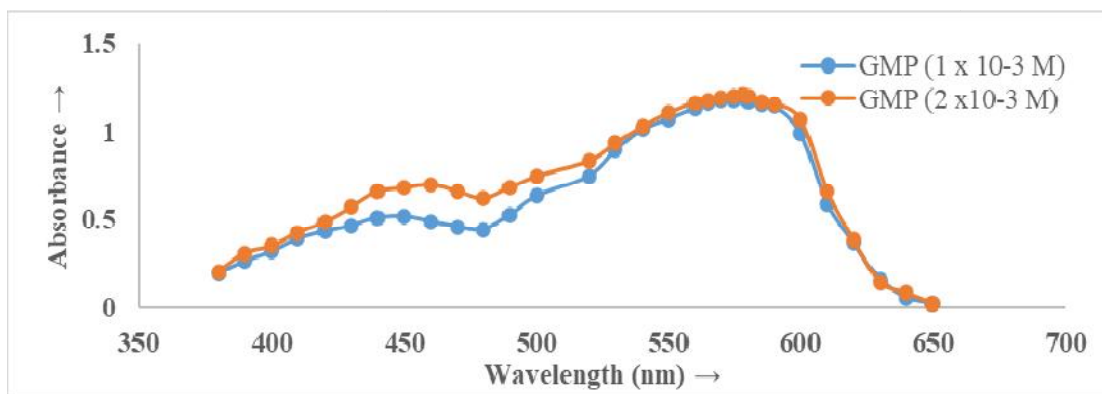


Fig. 3. Absorption spectra of the complex [GMP]: [BPB]

3.2 Composition of the Complex

The composition of the ion-pair complex of [GMP]: [CSR] and [GMP]: [BPB] was determined by Job's method and Mole ratio method.

3.2.1 Job's method

Different aliquots from 0.2 to 2.0 ml of GMP solution (1×10^{-3} M) and CSR solution (1×10^{-3} M) were transferred into the series of 10 ml volumetric flask and volume was made by chloroform. The continuous variation of GMP and dye solution was maintained. The absorbance of the complex was plotted against mole fraction of drug at their respective λ_{max} . Similar method was

followed with BPB. The plot of mole fraction and absorbance reached a maximum at 0.5 mole fraction [Figs. 4,5]. This indicates that ion-pair formation of a complex of GMP with dye in the ratio 1:1.

3.2.2 Mole ratio method

The stoichiometry of drug: dye was studied by molar ratio method. The absorbance of the complex was plotted against the [Drug]/[Dye] ratio. The plot shows maximum absorbance for Drug: Dye ratio 1:1 beyond which further addition of drug does not show change in absorbance [Figs. 6,7].

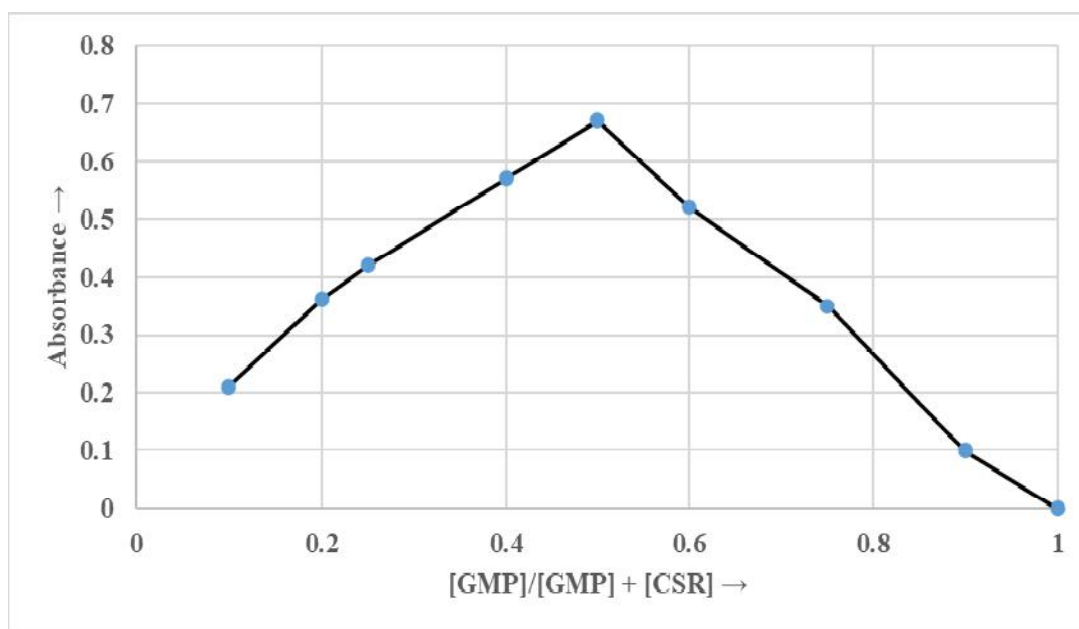


Fig. 4. Composition of ion – pair complex of Glimepiride with Cresol red by Job's method

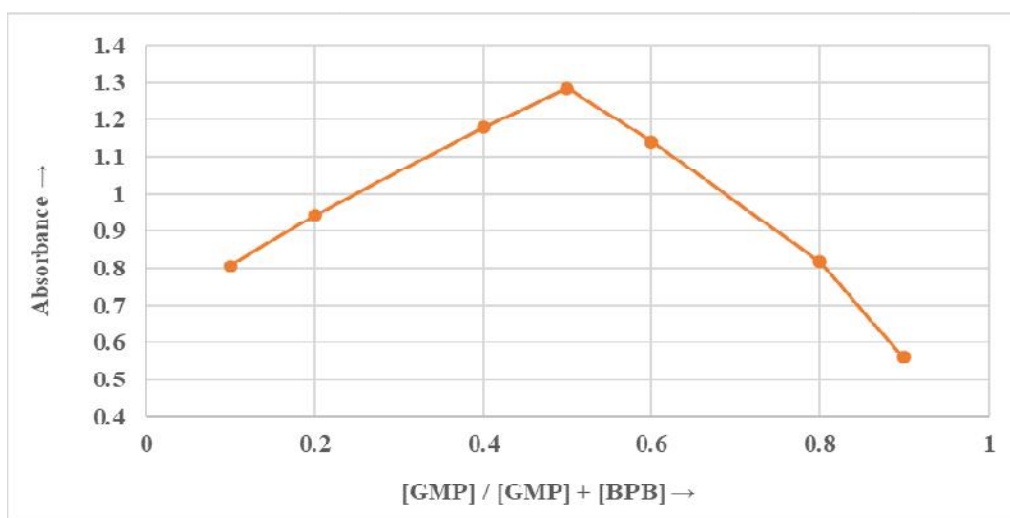


Fig. 5. Composition of the ion-pair complex of Glimepiride with Bromophenol blue by Job's method

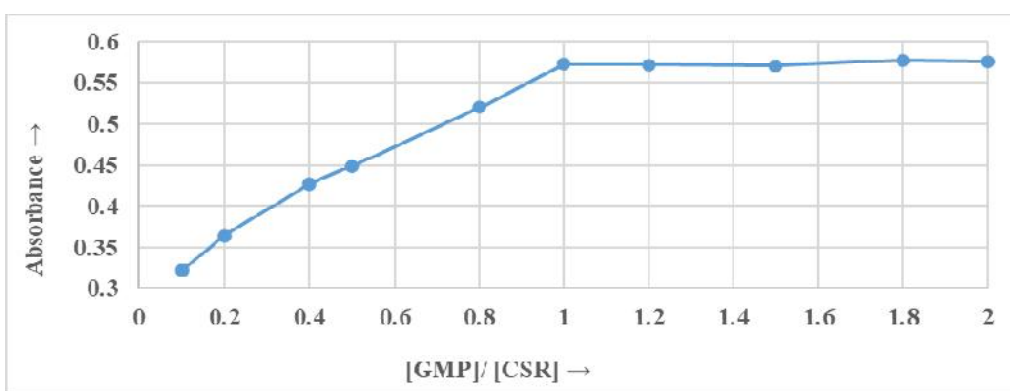


Fig. 6. Composition of the ion-pair complex of glimepiride with cresol red by Mole ratio method

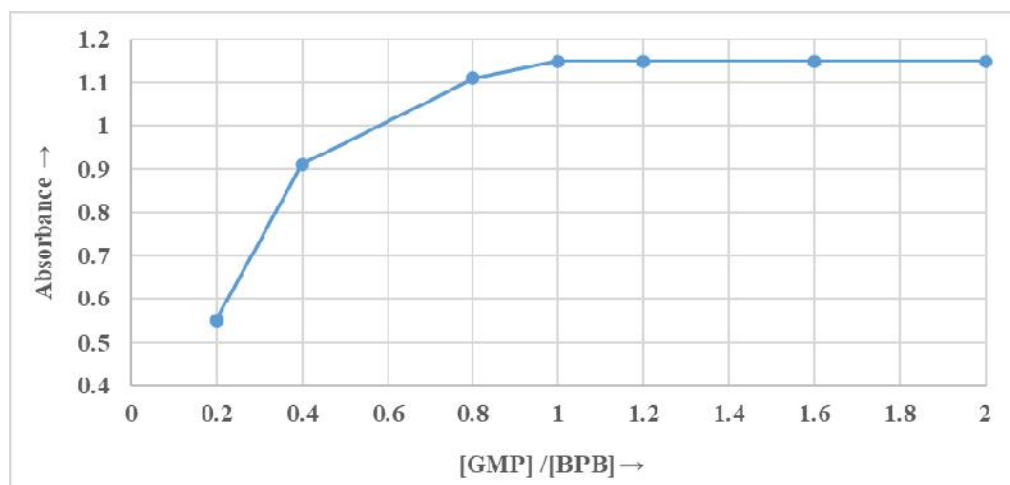


Fig. 7. Composition of ion – pair complex of glimepiride with bromophenol blue by Mole ratio method

3.3 Calibration Curve

Different aliquots ranging from 0.2 - 2.0 ml for method A and 0.2 to 1.0 ml for method B were transferred to a series of 10 ml volumetric flask. Same amount of dye solution (CSR or BPB) was added and the volume was made by chloroform. The calibration curve was constructed by plotting absorbances versus concentration of GMP at their respective maximum wavelengths [Figs. 8, 9]. The plot was linear over the range of 10 - 60 μgml^{-1} for method A and 2 - 20 μgml^{-1} for method B. The spectral

characteristics of the proposed method A and B is summarized in Table 1.

3.4 Reaction Mechanism

The anionic form of dye such as CSR and BPB form ion pair complex with the positively charged nitrogen containing molecule of glimepiride. The color of the complex is due to the opening of the lactoid ring and subsequent formation of quinoid group [14]. Glimepiride in the presence of a proton donor such as methanol form positively charged protonated Glimepiride.

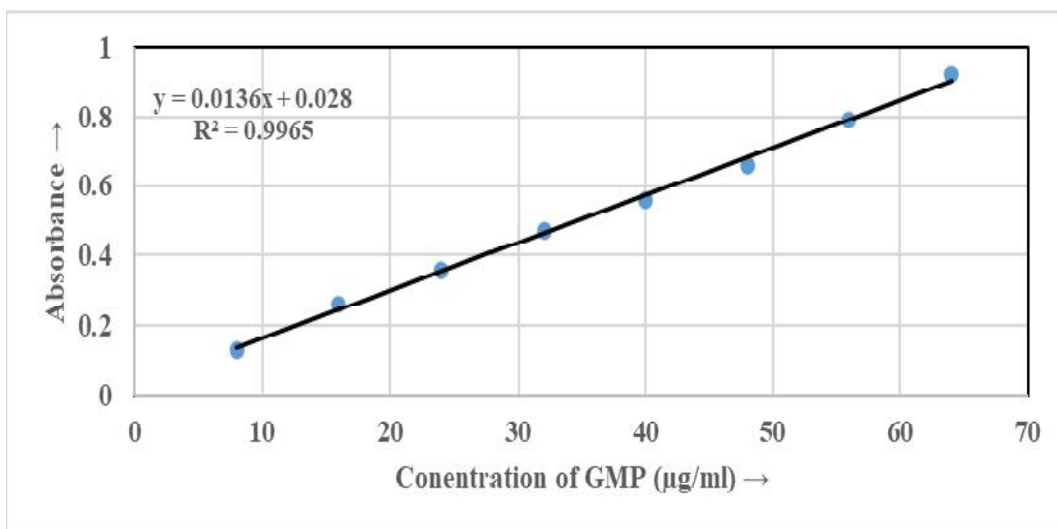


Fig. 8. Calibration curve of Glimepiride using Cresol red

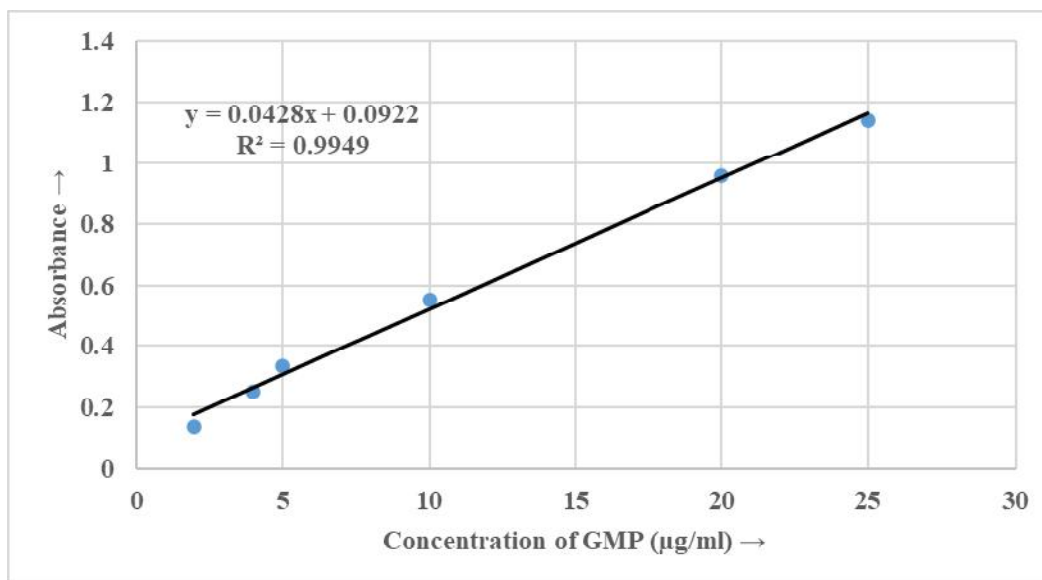


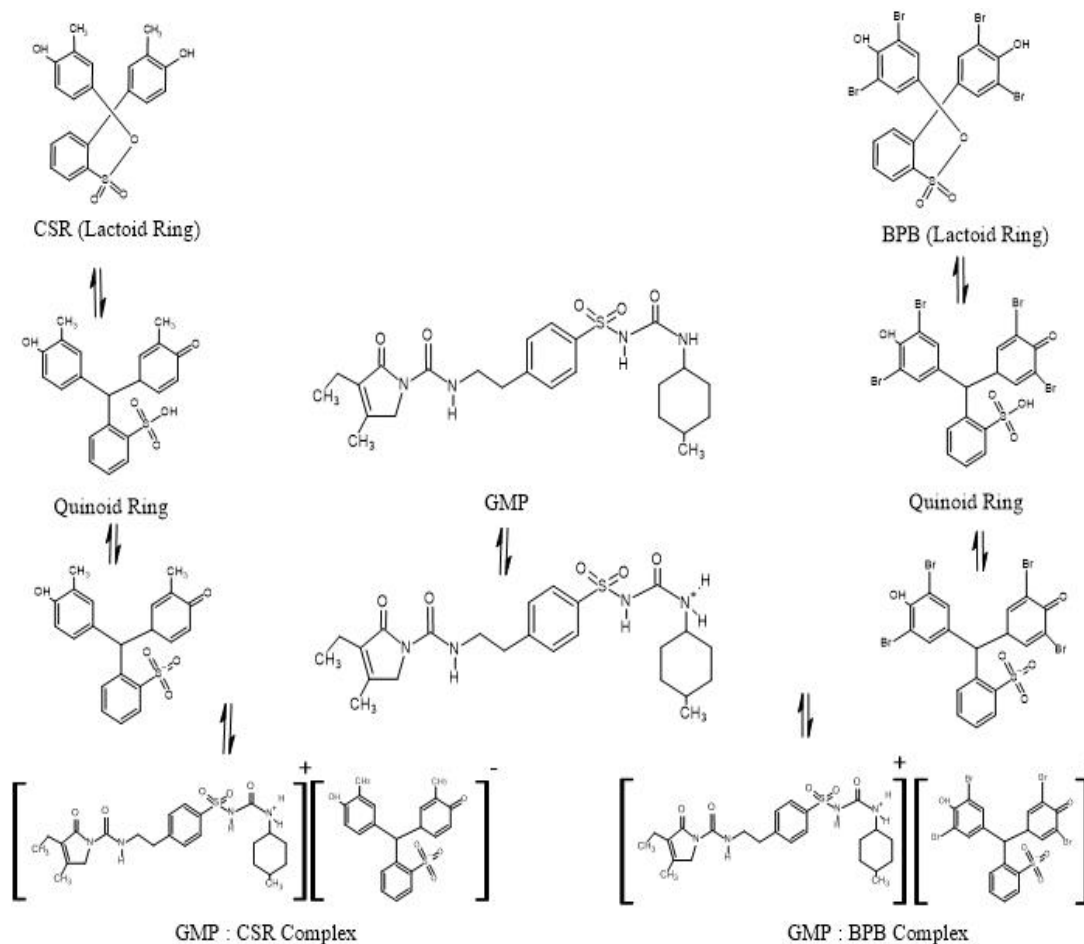
Fig. 9. Calibration curve of glimepiride using bromophenol blue

Table 1. The spectral parameters for the Spectrophotometric determination of GMP by ion-pair complex formation with CRS and BPB

Parameters	Method A	Method B
Maximum wavelength of absorbance (λ_{\max}) (nm)	450	578
Color of complex	Yellow	Red
Molar absorptivity (ϵ), ($\text{lmol}^{-1}\text{cm}^{-1}$)	6.6724×10^3	2.0999×10^4
Regression equation		
Slope	0.0136	0.0428
Intercept	0.0280	0.0922
Correlation coefficient (R^2)	0.9965	0.9949
Beer's law limit (μgml^{-1})	10 - 60	2 - 20
Sandell's sensitivity (μgcm^{-2} per 0.001 A)	0.0696	0.0177
Limit of Detection (LOD) (μgml^{-1})	0.1179	0.6173
Limit of Quantification (LOQ) (μgml^{-1})	0.0389	0.0203

It forms yellow and red colored ion-pair complexes with anionic form of Cresol red and Bromophenol blue, respectively. The ion-pair complex formed by two oppositely charged ions

held together as a single unit by an electrostatic binding. The possible mechanism of ion-pair complex formation of GMP with CSR and BPB is as shown in scheme [Fig. 10].

**Fig. 10. Reaction scheme of ion-pair complex formation of Glimepiride with Cresol red and Bromophenol blue**

3.5 Method Validation

The developed methods for estimation of Glimepiride have been validated in accordance with the International Conference on Harmonization (ICH) guidelines [15].

3.5.1 Linearity

Different aliquots of a standard stock solution (400 μgml^{-1}) of GMP were mixed in stoichiometric ratio of 1:1 with a dye solution in a series of 10 ml volumetric flask. They were similarly treated as discussed in calibration curve. Absorbances were plotted against the concentrations of GMP to obtain a calibration curve [Figs. 8, 9]. The linearity equations obtained for method A and B were $y = 0.0136x + 0.028$ and $y = 0.0428x + 0.0722$. The linearity range for method A was 10 - 60 μgml^{-1} and for method B was 2 - 20 μgml^{-1} .

3.5.2 Precision

The reproducibility of the proposed methods was determined by performing intraday and interday assay for five determinations on three consecutive days. The low percentage of RSD shows that the methods have good precision. Results of intraday and interday precision were expressed in % RSD [Tables 2, 3].

3.5.3 Accuracy

The accuracy of the method was determined by carrying recovery study of GMP by the standard addition method. The known amount of standard GMP (25%, 50% and 75%) was added to the prequantified sample solution within linearity range. The amount of GMP was calculated from the regression equation and the absorbance obtained. The results of accuracy were expressed in % recovery with standard deviation for five determination [Table 4].

3.5.4 Sensitivity

The sensitivity of the methods was expressed in terms of Sandell's sensitivity, LOD and LOQ using a standard calibration curve. The residual standard deviation of y-intercept of regression lines may be used to calculate LOD and LOQ using the equations,

$$\text{LOD} = \frac{3.3 \times \delta}{s} \quad \text{and} \quad \text{LOQ} = \frac{10 \times \delta}{s}$$

Where

δ = standard deviation of the intercepts of the regression line

s = slope of the calibration curve.

Table 2. Intraday precision for estimation of Glimepiride

Conc. of GMP (μgml^{-1})	Method A		Conc. of GMP (μgml^{-1})	Method B	
	Mean absorbance at 450 nm (n = 5)	% RSD		Mean absorbance at 578 nm (n = 5)	% RSD
40	0.574	0.1295	10	0.519	0.3140
40	0.573	0.3067	10	0.520	0.3697
40	0.571	0.1541	10	0.519	0.1655

Table 3. Interday precision for estimation of Glimepiride

Conc. of GMP (μgml^{-1})	Method A			Conc. of GMP (μgml^{-1})	Method B		
	Mean absorbance at 450 nm (n = 5)	% RSD			Mean absorbance at 578 nm (n = 5)	% RSD	
40	Day-1	0.572	0.3815	10	Day-1	0.520	0.2668
40	Day-2	0.568	0.4140	10	Day-2	0.517	0.3035
40	Day-3	0.563	1.1403	10	Day-3	0.515	0.3739

Table 4. Accuracy study for estimation of Glimepiride

	Conc. of GMP in sample (µg)	Level of std. addition	Amount of std. added (µg)	Amount recovered (µg) (n = 3)	Mean % Recovery	SD	% RSD
Method A	40	25%	10	50.196	100.39	±0.1121	0.2230
	40	50%	20	59.534	99.534	±0.1852	0.3111
	40	75%	30	69.755	99.650	±0.1535	0.2207
Method B	10	25%	2.5	12.511	100.088	±0.0357	0.285
	10	50%	5.0	14.855	99.033	±0.0468	0.315
	10	75%	7.5	17.319	98.966	±0.0234	0.1351

LOQ and LOD values for Glimepiride by method A and B are 0.1179, 0.6173 and 0.0389, 0.0203 respectively. The Sandell's sensitivity were 0.0696 and 0.0177 µgcm⁻² per 0.001 absorbance units for method A and B respectively [Table 1].

3.5.5 Robustness

The robustness of the proposed method was demonstrated by the constancy of the absorbance. The absorbance of the ion-pair complexes was studied by making deliberate changes in the experimental parameters such as temperature, stability and reaction time which indicate the robustness of the proposed method. The complexes were found to be stable more than 24 hours. The absorbance was measured and concentration of drug was calculated for five measurements [Table 5].

3.5.6 Specificity

In order to determine the specificity of the developed methods the absorption spectrum of

the ion pair complex of pure Glimepiride and commercial dosage form (Tablets) containing excipients of same concentration with the dye were scanned over 400 to 700 nm. The spectrum with pure Glimepiride was overlaid with the spectrum of commercial dosage form. The presence of commonly used excipients did not show any interference with the absorbance of Glimepiride. There was no change in the spectrum of drug in the presence of commonly used excipients in both the proposed methods. Absorption spectrum obtained for pure Glimepiride was almost identical with the spectra of marketed formulation in both the methods under investigation

3.5.7 Application to dosage form

The developed spectrophotometric methods were applied to determine Glimepiride in tablet dosage form available in local market. The amount of Glimepiride in single tablet was estimated from regression equation for five replicate determination [Tables 6,7].

Table 5. Robustness of the proposed method for estimation of Glimepiride

Parameters	Recovery	
	Method A	Method B
Temperature		
20 °C	99.4	99.8
25 °C	99.7	100.2
Reaction Time		
2.0 min	99.6	99.7
4.0 min	99.8	100.4
Stability		
10 h	100.1	99.8
20 h	100.3	100.1

Table 6. Estimation of Glimepiride in dosage form by method A

Formulation	Labelled claim (mg/tab)	Amount found (mg/tab.) (n=5)	% Recovery	SD	RSD
Amaryl Tab (Sanofi Aventis)	1.0	1.0023	100.23	±0.0004	0.0419
Dibiglim Tab (Novartis)	1.0	0.9872	98.72	±0.0044	0.4473
Glader - 1 Tab (Lupin)	1.0	0.9883	98.83	±0.0060	0.6055

Table 7. Estimation of Glimepiride in dosage form by method B

Formulation	Labelled Claim (mg/tab)	Amount found (mg/tab.) (n=5)	% Recovery	S.D.	RSD
Amaryl Tab (Sanofi Aventis)	1.0	0.9940	99.40	±0.0021	0.2104
Dibiglim Tab (Novartis)	1.0	0.9872	98.72	±0.0031	0.3140
Glader - 1 Tab (Lupin)	1.0	0.9875	98.75	±0.0023	0.2329

4. CONCLUSION

The developed spectrophotometric methods are simple, specific, sensitive, accurate and precise. Both the methods have very good linearity and can be used for determination of Glimepiride in pure and tablet dosage form. The analysis technique involved in these methods are very inexpensive and cost effective. No complicated extraction steps are involved at any stages of analysis. The recovery values were very high and the presence of excipients did not interfere with the absorbance of Glimepiride. Furthermore, in assay studies practical estimation of Glimepiride from formulations were in good agreement with their respective label claim with selective detection of Glimepiride. The developed methods can act as a tool for quality control of drug in analytical laboratories.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable.

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infrastructure facilities to carry out the proposed work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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