# 7Direct Spectrophotometric Determination of Glimepiride in Pure Form and Pharmaceutical Formulations Using Bromocresol Purple

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## ABSTRACT

A simple, direct and accurate spectrophotometric method has been developed for the determination of Glimepiride (GLM) in pure and pharmaceutical formulations by complex formation with bromocresol purple (BCP). The method involves the formation of a yellow ion-pair complex between BCP with glimepiride at pH<3,8; after reacting GLM with $Na_2CO_3$ to give $C_{24}H_{33}N_4H^+O_5NaS$ which is extracted by chloroform. The formed complex [GLM]: [ BCP] was measured at $\lambda_{max}$ 418 nm against the reagent blank prepared in the same manner. Variables were studied in order to optimize the reaction conditions. Molar absorptivity ($\varepsilon$) for complex was 20600 L.mol$^{-1}$.cm$^{-1}$. Beer's law was obeyed in the concentration range of 1.226 – 46.608 $\mu$g.mL$^{-1}$ in present of 5.0x10$^{-4}$ mol/l of BCP with good correlation coefficient ($R^2$= 0.9997). The relative standard deviation did not exceed 3.6%. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.15 and 0.46 $\mu$g. mL$^{-1}$, respectively. The proposed method was validated for specificity, linearity, precision and accuracy, repeatability, sensitivity (LOD and LOQ) and robustness. The developed method is applicable for the determination of GLM in pure and different dosage forms with average assay of 98.8 to 102.0% and the results are in good agreement with those obtained by the RP-HPLC reference method.

**Keywords.** Direct spectrophotometric method; Ion-pair complex; Glimepiride; Bromocresol purple.

## 1.  INTRODUCTION

Glimepiride (GLM) belongs to sulfonylurea oral anti diabetic and GLM is an anti-diabetic drug which is used for the treatment of diabetes. GLM is a white to yellowish-white, odorless, crystalline powder insoluble in water. It is chemically described as 1-[[p-[2-(3-ethyl-4methyl-2-oxo-3-pyrroline-1carboxamido) ethyl] phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl) urea ($C_{24}H_{34}N_4O_5S$) with a mol. mass of 490.62 g [1, 3], see Scheme 1.

Bromocresol purple $C_{21}H_{16}Br_2O_5S$ (BCP), acts as a weak acid in solution. It can thus be in protonated or deprotonated form, appearing yellow or purple, respectively, mol. mass 540.22 g [4], see scheme 2. Bromocresol purple has been used as a reagent to form ion pair complexes with drugs as gatifloxacin [5].

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**Scheme 1: Chemical structure of Glimepiride (GLM).**
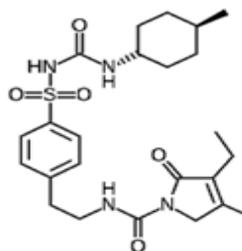
**Scheme 2:  Chemical structure of Bromo cresol purple (BCP).**



The objective of present study was to develop and validate an analytical method for quantitative determination and dissolution studies of glimepiride in tablets. The glimepiride shows absorption maxima at 225 nm and obeyed Beer's law in the range of 6.0 – 14.0 µg.mL$^{-1}$. The limit of detection and limit of quantitation were 0.06, and 0.17 µg.mL$^{-1}$ respectively. Percentage recovery of glimepiride for the proposed method ranged from 99.32 to 100.98% indicating no interference of the tablet excipients. It was concluded that the proposed method is simple, easy to apply, economical and used as an alternative to the existing spectrophotometric and non-spectrophotometric methods for the routine analysis of glimepiride in pharmaceutical formulations and in vitro dissolution studies. [6].

Two simple and sensitive visible spectrophotometric methods have been developed for the quantitative estimation of glimepiride from its tablet formulation. The developed methods are based on formation of chloroform extractable coloured complex of drug with methylene blue and safranine. The chloroform extracted complex of drug with methylene blue showed absorbance maxima at 652 nm and linearity was observed in the concentration range of 15-50 µg.mL$^{-1}$ (method-I), with safranine showed absorbance maxima at 536 nm and linearity was observed in the concentration range of 10-80 µg/mL (method-II). Results of analysis for both the developed methodswere validated statistically and by recovery studies. [7].

Various spectrophotometric methods [8-28] have been reported for the determination of Glimepiride in pure as well as in dosage forms. Most spectrophotometric methods employ extraction procedures. In this case, the extracted complexes were into an organic solvent, which is immiscible with water, and the concentration of the resulting complex in the organic phase is determined spectrophotometrically. The complex extraction technique has some difficulties and inaccuracies due to incomplete extraction or the formation of emulsions between the hydrocarbon solvent and the basic compound-containing solution. In response to the problems resulting from extraction of the complex, it is better to determine formed complex without extraction [29]. Also, none of the methods reported in the literatures is based on the formation of complex between BCP and GLM.

In this study, extraction-free spectrophotometric method for determination of GLM through ion-pair complex formation with BCP was developed.

## 2. MATERIALS AND METHODS

### 2.1. Equipment and Materials

Spectrophotometric measurements were made in Spectro scan 80 DV UV-VIS spectrophotometr with 1 cm quartz cells. An ultrasonic processor model Powersonic 405 was used to sonicate the sample solutions. The diluter pipette model DIP-1 (Shimadzu), having 100 µl sample syringe and five continuously adjustable pipettes covering a volume range from 20 to 5000 µL (model Piptman P, GILSON). Centrifuge (Centurion Scientific Ltd., Model: K2080-Manufactured in the United Kingdom) was used for preparation of the experimental solutions. SARTORIUS TE64 electronic balance was used for weighing the samples.

We used glimepiride (99.98%) was supplied by Chempi fine chemicals (INDIA), Metformin HCL (99.0%) and Rosuvastatin Calcium (98.6%). Bromocresol purple (99%) of analytical grade, chloroform Sigma-Aldrich and $Na_2CO_3$ extra pure were from MERCK. All solvents and reagents were analytical grade chemicals.

Tablet commercial formulations were used for the analysis of GLM. The pharmaceutical formulations subjected to the analytical procedure were:

(1) Amarium tablets, Racha Lab, Aleppo–Syria, each tablet contains 2 and 4 mg of GLM (Mfg. 5/2016, Exp. 5/2018, respectively).

(2) Amapiride tablets, Avenzor Pharmaceutical Industries, Damascus–Syria, each tablet contains 2 and 4 mg of GLM (Mfg. 7/2017, Exp. 7/2019, respectively).

### 2.2 Standard stock solutions

$1 \times 10^{-3}$ mol. $L^{-1}$ of pure GLM was prepared in chloroform. This solution was prepared by good mixing 12.27 mg of GLM with 0.05 g of $Na_2CO_3$, adding 0.1 mL $H_2O$, drying well in 105°C, after that it was dissolved in chloroform into a volumetric flask (25 mL) and diluted up to mark with chloroform. The solution was stored in dark bottles and kept in the refrigerator for not more than a week. The stock solution was further diluted daily just before the use to obtain working solutions of GLM in the concentration range 2.5-95 µM (1.226 – 46.608 µg. $mL^{-1}$ of GLM).

**Stock standard solution of Bromocresol purple (BCP) $1 \times 10^{-2}$ mol/l**: Accurately weighed 136.42 mg of BCP was dissolved in chloroform into a volumetric flask (25 mL) and diluted up to mark with chloroform.

### 2.3 Recommended Procedure

Aliquots of $1 \times 10^{-3}$ mol. $L^{-1}$ GLM solution (25, 50, 75, 100, 200, 300, 400, 500, 600, 750, 850 and 950 µL) containing 2.5, 5, 7.5, 10, 20, 30, 40, 50, 60, 75, 85 and 95 µM (1.226, 2.453, 3.680, 4.906, 9.812, 14.719, 19.625, 24.531, 29.437, 36.797, 41.703, and 46.608 µg. $mL^{-1}$) of GLM were transferred into a series of 10 mL calibrated volumetric flasks. Then 0.500 mL of BCP solution ($1 \times 10^{-2}$ mol. $L^{-1}$) was added. The volume was made up to the mark with solvent and the absorbance was measured at $\lambda_{max}$ 418 nm in chloroform, against a similar reagent blank.

### 2.4 Procedure for pharmaceutical formulations

Twenty tablets of each studied pharmaceutical formulation were weighed accurately, finely powdered and mixed well. An amount of the powder equivalent to the weight of one tablet was mixed well with 0.05 g of $Na_2CO_3$, adding 0.1 ml $H_2O$, drying well in 105°C, and solved in chloroform using ultrasonic for 15 min, 10 ml

of chloroform was added, filtered over a 10 ml flask and washed by the same solvent, then diluted to 10 ml with chloroform. This solution contains the following: 200 and 400 $\mu$g.mL$^{-1}$ of GLM for all studied pharmaceutical formulations contain 2 and 4 mg/tab, respectively.

Five solutions were prepared daily by diluting 1.0 mL from each stock solution of pharmaceutical formulations for contents: 2 or 4 mg/tab, then 0.4 ml from stock standard solution of BCP was added and adjusted the volume up to 10 ml with chloroform (these solutions contain 20 or 40 $\mu$g. mL$^{-1}$ of GLM respectively and $4\times10^{-4}$ mol. L$^{-1}$ of BCP; test solutions).

## 3. RESULTS AND DISCUSSION

### 3.1 The effect of solvent

The effect of the solvents (acetone, acetonitrile, dichloroethane, dichloromethane, chloroform and ethylacetate) on absorbance of reagent (BCP), formed complex [GLM][BCP] and the difference between them. It was found that chloroform solvent was the best, see Figure 1.
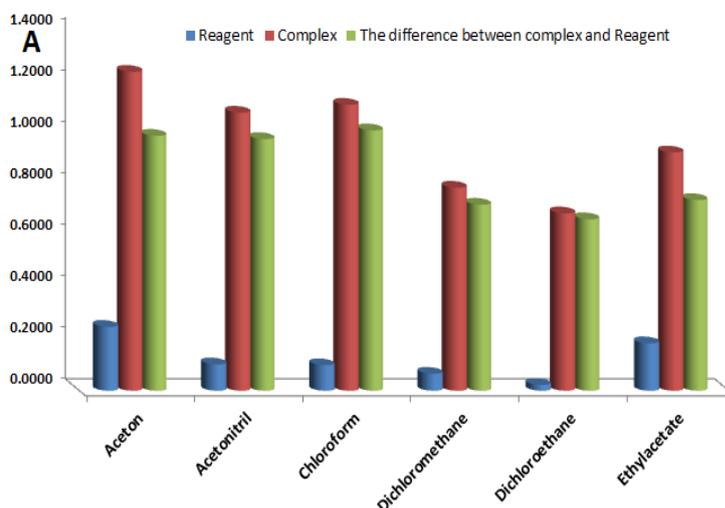


Fig. 1: Absorbance of reagent (BCP), formed complex [GLM]: [ BCP] and

difference between them (C$_{BCP}$ 2.5x10$^{-4}$ M, C$_{Complex}$ 5x10$^{-5}$ M, Blank is solvent, $\ell$ =1 cm).

### 3.2 Absorption Spectra

UV-Vis spectra of GLM, BCP and the formed complex GLM:BCP solutions in chloroform was obtained. GLM solutions do not absorb in the range 300-600 nm. BCP solutions have small absorption at $\lambda_{max}$ 418 nm ($\varepsilon\approx396$ L mol$^{-1}$ cm$^{-1}$ in chloroform). [GLM]: [BCP] complex solutions have maximum absorption at $\lambda_{max}$ 418 nm in chloroform, $\varepsilon$ for the complex was 20600 L mol$^{-1}$ cm$^{-1}$, see Figure 2 as example.

### 3.3 Optimization of Variables

The different experimental parameters affecting the spectrophotometric determination of GLM through ion-pair complex [GLM]: [BCP] formation with BCP in chloroform was studied in order to determine the optimal conditions for the determination of GLM.

## 3.4 The effect of time and temperature

The effect of time and temperature on the complex [GLM]: [BCP] formation was studied within the ranges 5-120 min and 15-45°C. It was found that the formed complex wasn't affected by time or temperature at those ranges.
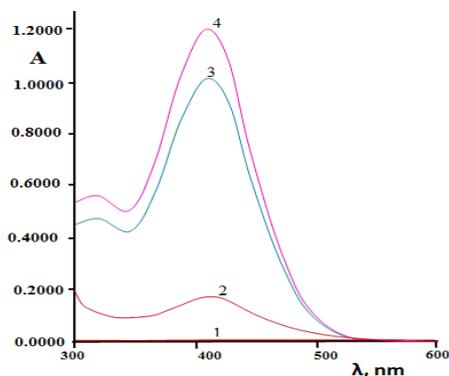
Fig.2. UV-Vis spectra in chloroform of: 1- $0.8 \times 10^{-4}$ mol.L$^{-1}$ of GLM; 2- $5.0 \times 10^{-4}$ mol.L$^{-1}$ of BCP; 3- $0.5 \times 10^{-4}$ mol.L$^{-1}$ ion-pair complex ($0.5 \times 10^{-4}$ mol.L$^{-1}$ of GLM with $5 \times 10^{-4}$ mol.L$^{-1}$ of BCP ); Blank is $5.0 \times 10^{-4}$ mol/l of BCP, 4- $0.5 \times 10^{-4}$ mol.L$^{-1}$ ion-pair complex ($0.5 \times 10^{-4}$ mol.L$^{-1}$ of GLM with $5 \times 10^{-4}$ mol.L$^{-1}$ of BCP ); Blank is chloroform, $\ell = 1$ cm.

## 3.5 The effect of BCP concentration

The effect of BCP concentration on complex [GLM]: [BCP] formation was investigated. It was observed that the absorbance of the formed complex increased coinciding with increasing the ratio of $C_{BCP}:C_{GLM}$ until the ratio (5:1), then slowly increased until the absorbance became a quasi-static at ratio more than 10.

## 3.6 Stoichiometric Relationship

### 3.6.1 The molar ratio method

The composition of GLM:BCP complex were determined by the molar ratio method and Job's method of continuous variation [30]. The stoichiometry of GLM: BCP complex was studied by molar ratio method according to following equation: $A_{max} = f$ ([BCP]/[GLM]) at $\lambda_{max}$ 418 nm in chloroform. It confirmed that the binding ratio of GLM: BCP complexes are equal to (1:1); where the concentration of GLM was constant (50 μM) and the concentrations of BCP changed from 0 to 250 μM, see Figure 3. The formation constant of the ion pair complex [GLM]: [ BCP] is $2.81 \times 10^8$ in chloroform.
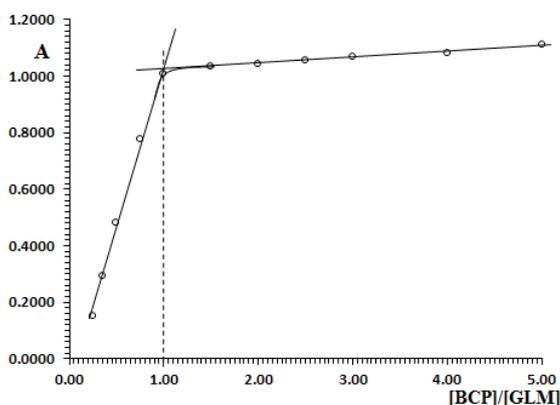
Fig.3. Molar ratio method to calculate binding ratio of GLM:BCP complex at $\lambda_{max}$ 418 nm in chloroform

([GLM]= 50 μM, blank is chloroform, $\ell = 1$ cm).

## 3.6.2 The Job's method

Continuous variation was utilized to check the composition of GLM:BCP complex at $\lambda_{max}$ 418 nm in chloroform. The absorbance of the complex in used solvent were plotted against the mole fraction [BCP]/([GLM]+ [ BCP]), where [GLM]+ [ BCP] =100 $\mu$M. The plot reached maximum value at a mole fraction of 0.5, see Figure 4. This indicated complex formation (GLM: BCP) in the ratio of (1:1). The formation constant of the ion- pair complex [GLM]: [BCP] is $2.44 \times 10^8$.
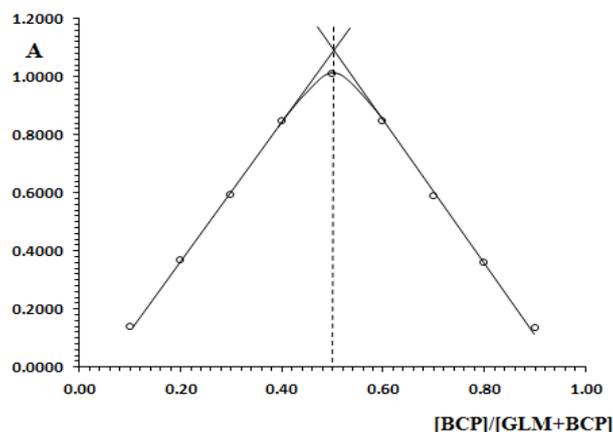


Fig.4. Job's method of continuous variation to calculate binding ratio of GLM: BCP complex at $\lambda_{max}$ 418 nm in chloroform ([GLM]+ [ BCP] =100$\mu$M, blank is chloroform, $\ell$ =1 cm).

## 3.7 Mechanism of reaction

Anionic dyes such as BCP form ion-pair complexes with the positively charged nitrogen-containing molecule. The colour of such dyes is due to the opening of lactoid ring and subsequent formation of quinoid group (deprotonated). Glimepiride ($C_{24}H_{34}N_4O_5S$) is reacted with $Na_2CO_3$ to give ($C_{24}H_{33}N_4H^+O_5NaS$), then dissolved in chloroform and forms yellow ion-pair complex with the dye at pH<3.8; (in pH>5.4 and alkaline solution BCP gives blue colour). Each drug-dye complex with two oppositely charged ions (positive on the drug and negative on the dye) behaves as a single unit held together by an electrostatic binding [14, 24-27]. The suggested mechanism of GLM- BCP ion-pair complex formation is shown in Scheme 3.
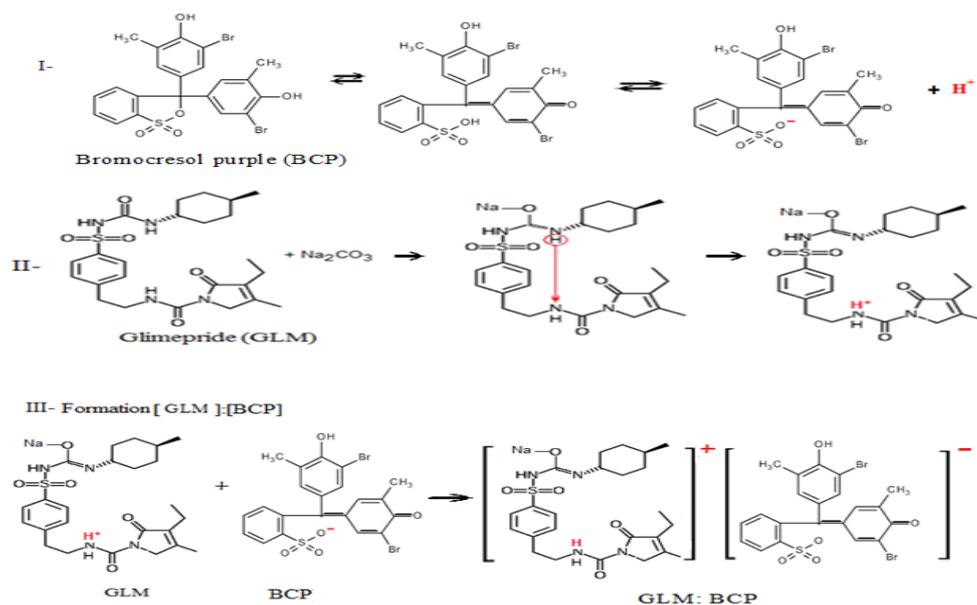


Scheme 3: The possible reaction mechanism of [GLM]: [BCP] complex formation.

### 3.8 Calibration curve

The calibration curve of GLM in pure form through complexation with BCP showed excellent linearity over concentration range of 1.226 – 46.608 µg. mL$^{-1}$ in presence of 5.0×10$^{-4}$ mol. L$^{-1}$ of BCP with good correlation coefficient (R$^2$= 0.9997) in chloroform. Regression equation at $\lambda_{max}$ was as the follows: y=0.0421x+0.0023 in chloroform. Figures 5 and 6 showed the spectra of [GLM]: [ BCP] complex in presence of 5.0×10$^{-4}$ M of BCP as example. The spectra characteristics of the method such as the molar absorptivity ($\varepsilon$), Beer's law, regression equation at $\lambda_{max}$ (y=a.x+b); where y=absorbance, a=slope, x=concentration of GLM by µg. mL$^{-1}$, b=intercept, the correlation coefficient, limit of detection (LOD) and limit of quantification (LOQ) and the optimum conditions for spectrophotometric determination of GLM through ion-pair complex formation using BCP in chloroform is summarized in Table 1.
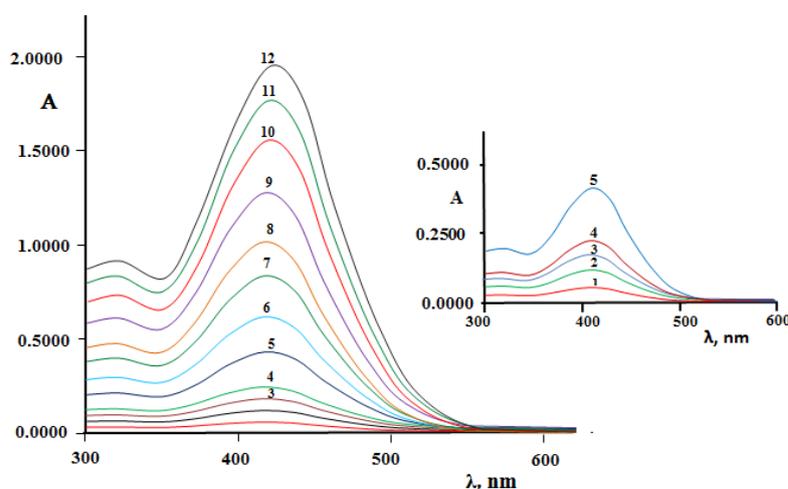


Fig.5. Spectra of [GLM]: [BCP] complex in presence of 5.0×10$^{-4}$ M of BCP; where C$_{GLM}$ as the follows: 1.226, 2.453, 3.680, 4.906, 9.812, 14.719, 19.625, 24.531, 29.437, 36.797, 41.703 and 46.608 µg. mL$^{-1}$ {Blank is BCP solution in chloroform 5.0×10$^{-4}$ M; $\ell$ = 1cm}.

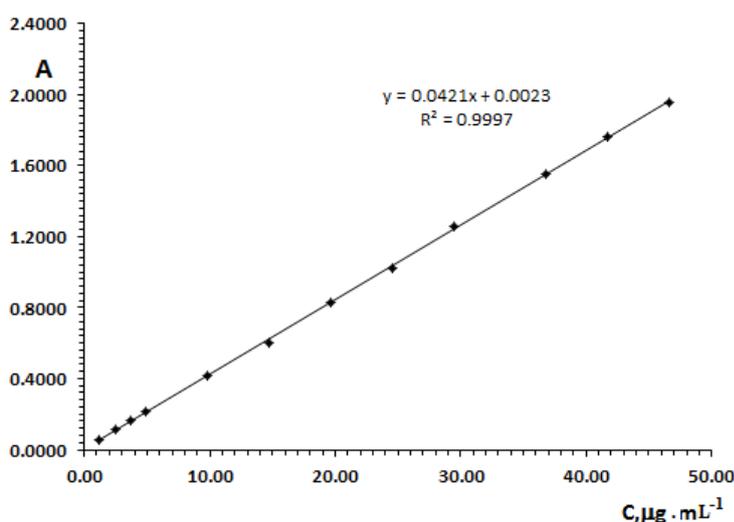

Fig. 6: Calibration curve for determination of GLM according to optimal conditions at λmax 418 nm (in present of 5.0×10$^{-4}$ M of BCP) where GLM: 1.226 – 46.608 µg.mL$^{-1}$ {Blank is BCP solution in chloroform 5.0×10$^{-4}$ M; $\ell$ = 1 cm}.

Table 1. The parameters established for spectrophotometric determination of GLM by complex formation with BCP in chloroform.

| Parameters | Operating values |
|---|---|
| $\lambda_{max}$ of GLM: CPB complex, nm | 418 |
| Beer's Law Limit, for $C_{GLM}$ by $\mu M$ | 2.5-95 |
| Beer's Law Limit, for $C_{GLM}$ by $\mu g.\ mL^{-1}$ | 1.226 – 46.608 |
| Molar absorptivity of [GLM]: [BCP] complex ($\varepsilon$),$L.mol^{-1}.cm^{-1}$ | 20600 |
| Regression equation for [GLM]: [BCP] at $\lambda_{max}$ =418 nm: | |
| Slope | 0.0421 |
| Intercept | 0.0023 |
| Correlation coefficient ($R^2$) | 0.9997 |
| LOD for $C_{GLM}$ by $\mu g.\ mL^{-1}$ | 0.15 |
| LOQ for $C_{GLM}$ by $\mu g.\ mL^{-1}$ | 0.46 |
| RSD% | 3.6 |
| $C_{BCP}$: $C_{GLM}$, M | ≥5 |
| Stability | 24 h |
| Temperature of solution | 20±5°C |

n=5, t=2.776.

## 4. Analytical results

Spectrophotometric determination of GLM through complexation with BCP in chloroform within optimal conditions using calibration curve was applied. The results, summarized in Table 2, showed that the determined concentration of GLM was rectilinear over the range of 1.226 – 46.608 $\mu g.\ mL^{-1}$, with relative standard deviation (RSD) not more than 3.6%. The results obtained from the developed method have been compared with the official RP-HPLC method [30] and good agreement was observed between them.

Table 2: Spectrophotometric determination of GLM through complex formation with BCP within optimal conditions using calibration curve in chloroform.

| $X_i$, $\mu g.\ mL^{-1}$ (Taken) | *$\overline{X}$ ±SD, $\mu g.\ mL^{-1}$ (Found) | $\overline{x} \pm \dfrac{t.SD}{\sqrt{n}}$ $\mu g.\ mL^{-1}$ | RSD% | *X , $\mu g.\ mL^{-1}$ RP-HPLC [31] |
|---|---|---|---|---|
| 1.226 | 1.235±0.0445 | 1.235± 0.0552 | 3.6 | 1.216 |
| 2.453 | 2.553±0.087 | 2.553± 0.108 | 3.4 | 2.436 |
| 3.680 | 3.824±0.122 | 3.824± 0.152 | 3.2 | 3.721 |
| 4.906 | 5.064±0.152 | 5.064± 0.189 | 3.0 | 4.926 |
| 9.812 | 9.812±0.294 | 9.812± 0.365 | 3.0 | 9.925 |
| 14.719 | 14.316±0.429 | 14.316± 0.533 | 3.0 | 14.897 |
| 19.625 | 19.637±0.550 | 19.637± 0.683 | 2.8 | 18.782 |
| 24.531 | 24.133±0.627 | 24.133± 0.779 | 2.6 | 25.214 |
| 29.437 | 29.732±0.743 | 29.732± 0.923 | 2.5 | 29.890 |
| 36.797 | 36.805±0.883 | 36.805± 1.097 | 2.4 | 38.125 |
| 41.703 | 41.722±0.960 | 41.722± 1.191 | 2.3 | 42.250 |
| 46.608 | 46.456±1.022 | 46.456±1.2688 | 2.2 | 46.889 |

* n=5, t= 2.776.

## 5. Applications

The developed spectrophotometric method was applied to determine GLM in some pharmaceutical preparations through complex formation by BCP in chloroform according to the optimal conditions. The results of quantitative analysis for GLM in pharmaceutical preparations were summarized in Table 3. The proposed method was simple, direct, specific and successfully applied to the determination of GLM in pharmaceuticals without any interference from excipients. Average recovery ranged between 98.8 to 102.0%. The results obtained by this method agree well with the contents stated on the labels and were validated by RP-HPLC method [31].

Table 3: Determination of GLM, in some Syrian pharmaceutical preparations using spectrophotometric method through complex formation with BCP in chloroform, $\lambda_{max}$ 418 nm.

| Tablet dosage form | Label Claim of GLM, mg/tab. | *Mean ±SD (GLM)، mg/tab. | RSD% | Assay % | *Mean ±SD (GLM)، mg/tab. by RP-HPLC [31] | * Assay %, by RP-HPLC [31] |
|---|---|---|---|---|---|---|
| Amarium | 2 | 1.990±0.062 | 3.1 | 99.5 | 2.016±0.042 | 100.8 |
| | 4 | 3.972±0.111 | 2.8 | 99.3 | 3.996±0.056 | 99.9 |
| Amapiride | 2 | 2.040±0.061 | 3.0 | 102.0 | 2.030±0.038 | 101.5 |
| | 4 | 3.952±0.115 | 2.9 | 98.8 | 3.960±0.055 | 99.0 |

**\*** n=5.

## 6. METHOD VALIDATION

The developed method for simultaneous estimation of GLM has been validated in accordance with the International Conference on Harmonization guidelines (ICH) [32].

### 6.1 Selectivity and Specificity

Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, standard solution of GLM, commercial product solution and blank solutions were analyzed. The results of the tests proved that the components other than the drug did not produce any interfere. The specificity of the method was ascertained by analyzing standard GLM in presence of excipients. There was no interference from most of the common excipients.

### 6.2 Linearity

Several aliquots of standard stock solution of GLM were taken in different 10 ml volumetric flask and diluted up to the mark with chloroform such that their final concentrations were 1.226 – 46.608 µg. mL$^{-1}$ for GLM. Absorbance was plotted against the corresponding concentrations to obtain the calibration graph, see Figure 5 and Table 3. Linearity equations obtained were y=0.042x+0.0023 for the range 1.226 – 46.608 µg. mL$^{-1}$ ($R^2$=0.9997).

### 6.3 Precision and Accuracy

The precision and accuracy of proposed method was checked by recovery study by addition of standard drug solution to pre-analyzed sample solution at three different concentration levels (80%, 100% and 120%) within the range of linearity for GLM. The basic concentration level of sample solution selected for spiking of the GLM standard solution was 14.719 µg. mL$^{-1}$. The proposed method was validated statistically and through

recovery studies and was successfully applied for the determination of GLM in pure and dosage forms with percent recoveries ranged from 99.8% to 102.2%, see Table 4.

Table 4: Results of recovery studies (n=5).

| Level | % Recovery |
|-------|------------|
| 80%   | 100.1      |
| 100%  | 99.8       |
| 120%  | 102.2      |

## 7.4 Repeatability and Robustness

The repeatability was evaluated by performing 10 repeat measurements for 14.719 µg. mL$^{-1}$ of GLM using the studied spectrophotometric method under the optimum conditions. The found amount of GLM ($\bar{\mathbf{x}} \pm$ SD) 14.338 ± 0.420 µg. mL$^{-1}$ and the percentage recovery was found to be 99.7 ± 2.85. These values indicate that the proposed method has high repeatability for GLM analysis. The robustness of the method adopted is demonstrated by the constancy of the absorbance with the deliberated minor change in the experimental parameters such as the change in the concentration of excipients, $C_{BCP}$: CGLM (10±5%), temperature (20±5$^o$C), stability (24±0.5 h) and reaction time (5±1 min), see Table 5 which indicates the robustness of the proposed method. The absorbance was measured and assay was calculated for five times.

Table 5: Robustness of the proposed spectrophotometric method.

| Experimental parameter variation | Average recovery (%)* | |
|---|---|---|
| | $C_{GLM}$ | |
| | 4.906 µg.mL$^{-1}$ | 36.797 µg.mL$^{-1}$ |
| Temperature | | |
| 15$^o$C | 99.6 | 99.9 |
| 25$^o$C | 100.7 | 100.9 |
| Stability | | |
| 23.5 h | 102.4 | 100.0 |
| 24.5 h | 102.6 | 100.5 |
| Reaction time | | |
| 4.0 min | 99.8 | 99.7 |
| 6.0 min | 102.7 | 101.2 |

* n=5.

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## 6.5 Sensitivity (LOD and LOQ)

The sensitivity of the method was evaluated by determining the LOD and LOQ. The values of LOD and LOQ for GLM are 0.15 and 0.46 µg. mL$^{-1}$, respectively.

## 6.6 The homogenization of tablets

The homogenization of tablets in terms of the weight and the amount of drug was studied. We found that the mean weight and amount drug in the tablets was 0.1793 ± 0.0030 g (i.e. ±1.67%), 0.1794 ± 0.0023 g (i.e. ±1.17%) for Amarium tablets (2 and 4 mg/tab) and 0.1005 ± 0.0015g (i.e. ±1.49%) and 0.0993 ± 0.0012 g (i.e. ±1.21%) Amapiride tablets (2 and 4 mg/tab), respectively. While the mean amount drug in the tablets was 1.999±0.070 mg/tab (i.e. ±3.5%) and 3.965±0.119 mg/tab (i.e. ±3.0%) for Amarium tablets (2 and 4 mg/tab) and 2.036±0.069 mg/tab (i.e. ±3.4%) and 3.945±0.122 mg/tab (i.e. ±3.1%) for Amapiride tablets (2 and 4 mg/tab), respectively; which shows that homogeneity of tablets is good.

## 6.7 Interferences

Metformin HCL up to 1000 mg with 2 mg of glimepiride does not interfere, but Pioglitazone HCL and Rosuvastatin Calcium interfere.

## 7. CONCLUSION

The developed spectrophotometric method is simple, direct (extraction-free) and cost-effective for the determination of GLM in pure and tablet dosage forms. This method is based on formation of ion-pair complex between GLM and BCP in chloroform ([GLM]: [BCP]). Beer's law in the optimum experimental conditions using [GLM]: [BCP] complex is valid within a concentration range of 1.226 – 46.608 µg/ml. The developed method is applied for the determination of GLM in pure and its commercial tablets without any interference from excipients with average assay of 98.8 to 102.0%.

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