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Original Article

SPECTROPHOTOMETRIC DETERMINATION AND VALIDATION OF GLIMEPIRIDE IN PURE AND TABLET DOSAGE FORMS THROUGH ION-PAIR COMPLEX FORMATION USING BROMOCRESOL GREEN

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ABSTRACT

Objective: 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 green (BCG).

Methods: The method involves the formation of a yellow ion-pair complex between bromocresol green reagent with glimepiride ($C_{24}H_{34}N_4O_5S$); after reacted it with Na_2CO_3 to give $C_{24}H_{33}N_4H^+O_5NaS$ in chloroform at pH≤3.8.

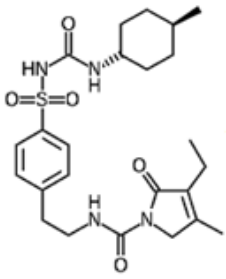
Results: The formed complex was measured at λ_{max} 416 nm against the reagent blank prepared in the same manner. Variables were studied in order to optimize the reaction conditions. Beer's law was obeyed in the concentration range of 0.981-9.812 µg/ml in the present of $1x10^{-4}$ mol/l of (BCG) and 9.812-58.874 µg/ml in the present of $1x10^{-3}$ mol/l of (BCG) with good correlation coefficient (R^2= 0.9992 and R^2= 0.9997, respectively). The relative standard deviation did not exceed 3.0%. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.088 and 0.29 µg/ml, respectively. The proposed method was validated for specificity, linearity, precision and accuracy, repeatability, sensitivity (LOD and LOQ), and robustness with average recovers 98.9 to 102.4%.

Conclusion: The developed method is applicable for the determination of glimepiride in pure and different dosage forms with average assay of marketed formulations 97.8 to 102.4% and the results are in good agreement with those obtained by the RP-HPLC reference method.

Keywords: Direct spectrophotometric method, Glimepiride, Bromocresol green, Ion-pair complex

INTRODUCTION

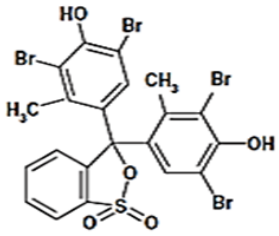
Glimepiride (GLM) belongs to sulfonylurea oral anti-diabetic. GLM is a white to yellowish-white, odorless, crystalline powder insoluble in water. It is chemically described as 1-[[p-[2-(3-ethyl-4methyl-2-oxo-3-pyrroline-1carboxamido) ethyl] phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl) urea ($C_{24}H_{34}N_4O_5S$)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



Scheme 1: Chemical structure of glimepiride

Bromocresol green ($C_{21}H_{14}Br_4O_5S$), mol. mass 698.01 g, is a dye of the triphenylmethane family (triaryl methane dyes) [3], see scheme 2. Bromocresol green has been used as a reagent to form ion pair complexes with drugs [4, 5].

Various spectrophotometric methods [6-20] have been reported for the determination of glimepiride in pure as well as in dosage forms. Most spectrophotometric methods employ ion-pair extraction procedures. In this case, the ion-pair complex was extracted into an organic solvent, which is immiscible with water, and the concentration of the resulting ion-pair in the organic phase is determined spectrophotometrically. The ion-pair 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.



Scheme 2: Chemical structure of bromocresol green ($C_{21}H_{14}Br_4O_5S$)

In response to the problems resulting from the extraction of the ion-pair complex, it is better to determine formed ion pair complex without extraction [17]. Also, none of the methods reported in the literature is based on the formation of a complex between bromocresol green and GLM.

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

MATERIALS AND METHODS

Instruments and apparatus

Spectrophotometric measurements were made in Spectro scan 80 DV UV-VIS spectrophotometer with 1 cm quartz cells. An ultrasonic processor model Power sonic 405 was used to sonicate the sample

solutions. The diluted 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 the preparation of the experimental solutions. SARTORIUS TE64 electronic balance was used for weighing the samples.

Reagents

Glimepiride (99.98%) was supplied by Chempi fine chemicals (INDIA). Bromocresol green (97%) of analytical grade, chloroform and Na_2CO_3 extra pure were from MERCK. All solvents and reagents were analytical grade chemicals.

Stock standard solution of bromocresol green ($1x10^{-2}$ mol/l)

Accurately weighed 179.9 mg of BCG was dissolved in chloroform into a volumetric flask (25 ml) and diluted up to mark with chloroform.

Stock standard solution of GLM **($1x10^{-3}$ mol/l)**

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 ^oC, after that dissolving in chloroform, filtering over a 25 ml flask and washing by the same solvent, then diluting to 25 ml with chloroform.

Working standard solutions of glimepiride

The stock solution was further diluted daily just before the use to obtain working solutions of GLM in the concentrations: 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 80, 100 and 120 µM (0.981, 1.962, 2.944, 3.925, 4.906, 7.359, 9.812, 14.718, 19.625, 24.530, 29.437, 39.249, 49.062 and 58.874 µg/ml) by transferring different aliquots from stock standard solution: 20, 40, 60, 80, 100, 150, 200, 300, 400, 500, 600, 800, 1000 and 1200 µl into 10 ml volumetric flasks, then 1 ml from stock standard solution of BCG was added, diluted to 10 ml with chloroform.

Sample preparation

Commercial formulations (as a tablet) were used for the analysis of glimepiride. The pharmaceutical formulations subjected to the analytical procedure were:

(1) Glimaryl tablets, Asia pharma Co., Aleppo–SYRIA (Mfg. 02/2015, Exp. 02/2018), each tablet contains: 2 and 4 mg of GLM (2) Amarium tablets, Racha lab., Aleppo–SYRIA (Mfg. 01/2014, Exp. 02/2017), each tablet contains: 2 and 4 mg of GLM.

Stock solutions of pharmaceutical formulations

20 tablets of each studied pharmaceutical formulation were weighed accurately, crushed to a fine powder 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 and solved in chloroform using ultrasonic, 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 follows: 200 and 400 µg/ml of GLM for all studied pharmaceutical formulations contain 2 and 4 mg/tab, respectively.

Working solutions of pharmaceutical

Five solutions were prepared daily by diluting 1.000 ml from each stock solution of pharmaceutical formulations for contents: 2 and 4 mg/tab, then adding 1 ml from stock standard solution of BCG and adjusting the volume up to 10 ml with chloroform (these solutions contain 20 or 40 µg/ml of GLM respectively; test solutions).

Procedure

A solution (10 ml) containing an appropriate concentration of GLM (or working solutions of pharmaceuticals) with appropriate amount of bromocresol green in chloroform was ready for spectro-photometric measurement at λ_{max} 416 nm.

RESULTS AND DISCUSSION

The different experimental parameters affecting the spectro-photometric determination of glimepiride through ion-pair complex formation with bromocreol green in chloroform were studied in order to determine the optimal conditions for the determination of GLM.

Spectrophotometric results

UV-Vis spectra of GLM, BCG and the formed complex GLM: BCG solutions (using chloroform as blank or $1x10^{-3}$M of BCG in chloroform) were obtained. GLM solutions do not absorb in the range 300-600 nm. Bromocresol green (BCG) solutions have small absorption at λ_{max} 416 nm (ε=430 l. mol^{-1}. cm^{-1}). BCG: GLM complex solutions have maximum absorption at λ_{max} 416 nm, (ε=12000 l. mol^{-1}. cm^{-1}), see fig. 1.

The effect of time and temperature

The effect of time and temperature on the complex formation was studied within the ranges 5-120 min and 15-30 ^oC. It was found that the formed complex wasn't affected by time or temperature at those ranges.

The effect of BCG concentration

The effect of BCG concentration on complex formation was investigated. It was observed that the absorbance of the formed complex increased coinciding with increasing the ratio of C_{BCG}: C_{GLM} until the ratio (1:1), then stayed quasi-constant.

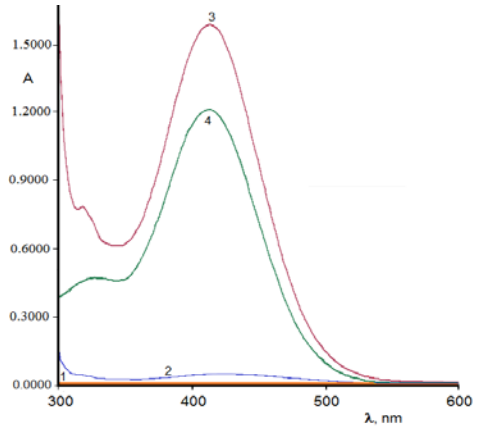


Fig. 1: UV-Vis spectra in chloroform of: 1-1.0x10^{-4} mol/l of GLM; 2-1x10^{-4} mol/lof BCG; 3,4-1.0x10^{-4} mol/l ion-pair complex (1.0x10^{-4} mol/l of GLM with 1.0x10^{-3} mol/l of BCG); 1-3 blank is chloroform and 4-blank is 1x10^{-3}M of BCG in chloroform, ℓ =1 cm

Composition of GLM: BCG complex

The composition of GLM: BCG complex was determined by the molar ratio method and Job's method of continuous variation.

Molar ratio method

The stoichiometry of GLM: BCG complex was studied by molar ratio method according to the following equation: A_{max}= f([GLM]/[BCG]). It confirmed that the binding ratio of GLM: BCG complex is equal to (1:1); where the concentration of BCG was constant 50 µM and the concentrations of GLM changed from 0 to 100 µM

(fig. 2). The formation constant of the ion pair complex is 1.12x10^7.

Job's method of continuous variation

Continuous variation was utilized to check the composition of GLM: BCG complex. The absorbance of the complex was plotted against the mole fraction [GLM]/[GLM]+[BCG]. The plot reached a maximum value at a mole fraction of 0.5 (fig. 3). This indicated complex formation (GLM: BCG) in the ratio of 1:1. The formation constant of the ion pair complex is 1.03x10^7.

The optimum conditions for spectrophotometric determination of glimepiride through ion-pair complex formation using bromocresol green in chloroform are shown in table 1.

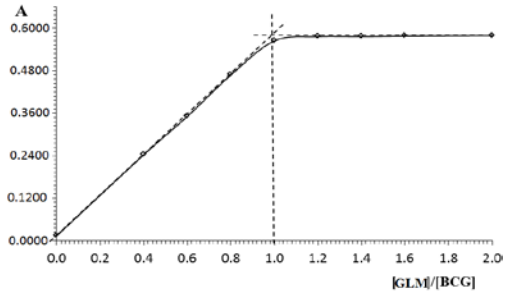


Fig. 2: Molar ratio method to calculate binding ratio of GLM: BCG complex at λ=416 nm ([BCG]= 50 µM, blank is chloroform, ℓ =1 cm)

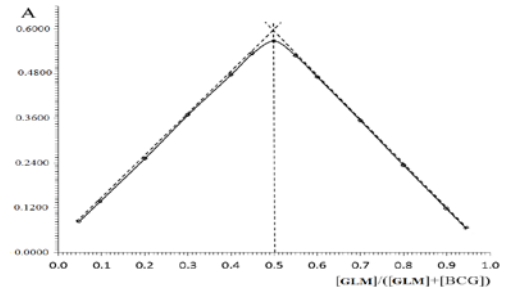


Fig. 3: Job's method of continuous variation to calculate binding ratio of GLM: BCG complex at λ 416 nm ([BCG]+[GLM]=100 µM, blank is chloroform, ℓ =1 cm)

Table 1: The optimum conditions for spectrophotometric determination of GLM by complex formation with BCG in chloroform

Parameters	Operating modes
Temperature of solution	20±5°C
C_{BCG}: C_{GLM}, M	≥5
Solvent	chloroform
Stability	24 h
λ_{max} of GLM: BCG complex	416 nm
Light path (ℓ)	1.0 cm
Spectra range	300–600 nm

Mechanism of reaction

Anionic dyes such as BCG 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 BCG 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 [8, 21-24]. The suggested mechanism of GLM-BCG ion-pair complex formation is shown in Scheme 3.

Calibration curve

The calibration curve of GLM in pure form through complexation with BCG showed excellent linearity over the concentration range of 2.0-120.0 µM (0.981–58.874 µg/ml). Regression equations at λ_{max} 416 nm were as the follows:

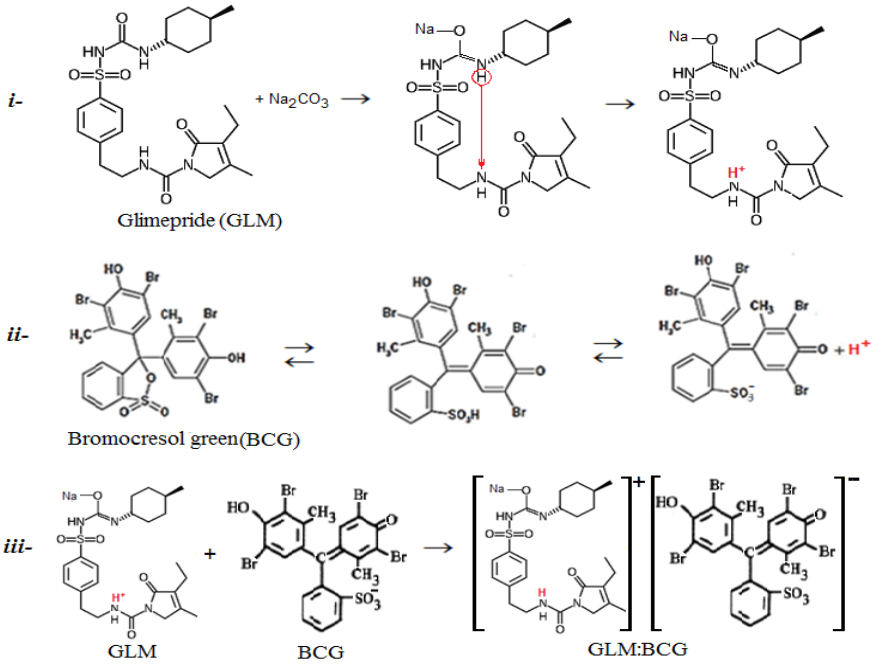
$$y=0.0244x+0.0011 \quad (I)$$

$$y=0.0245x+0.0028 \quad (II)$$

For concentrations of GLM 0.981-9.812 µg/ml and 9.812-58.874 µg/ml, respectively, (fig. 4&5). The spectra characteristics of the method such as the molar absorptivity (ε), Beer's law, regression equation at λ_{max} 416 nm (y=a.x+b); where y=absorbance, a=slope, x=concentration of GLM in µM or µg/ml,b=intercept, the correlation coefficient, limit of detection (LOD) and limit of quantification (LOQ) are summarized in table 2.

Analytical results

Spectrophotometric determination of GLM through complexation with BCG in chloroform within optimal conditions using calibration curve was applied. The results, summarized in table 3, showed that the determined concentration of GLM was rectilinear over the range of 2.0 to 120.0 µM or 0.981 to 58.874 µg/ml with relative standard deviation (RSD) not more than 3.0%. The results obtained from the developed method have been compared with the official RP-HPLC method [25] and good agreement was observed between them.



Schème 3: Mechanism of GLM: BCG complex formation

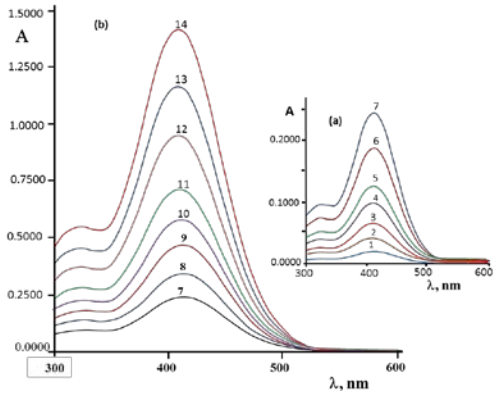


Fig. 4: Spectra of GLM: BCG complex in present 1×10^{-4} M of BCG (a) and 1×10^{-3} M of BCG (b); where C_{GLM} as the follows: 1-0.981, 2-1.962, 3-2.944, 4-3.925, 5-4.906, 6-7.359, 7-9.812, 8-14.718, 9-19.625, 10-24.530, 11-29.437, 12-39.249, 13-49.062 and 14-58.874 µg/ml, {Blank is BCG solution in chloroform: 1x10^{-4}M (1-7) and 1x10^{-3}M (7-14); ℓ = 1 cm}

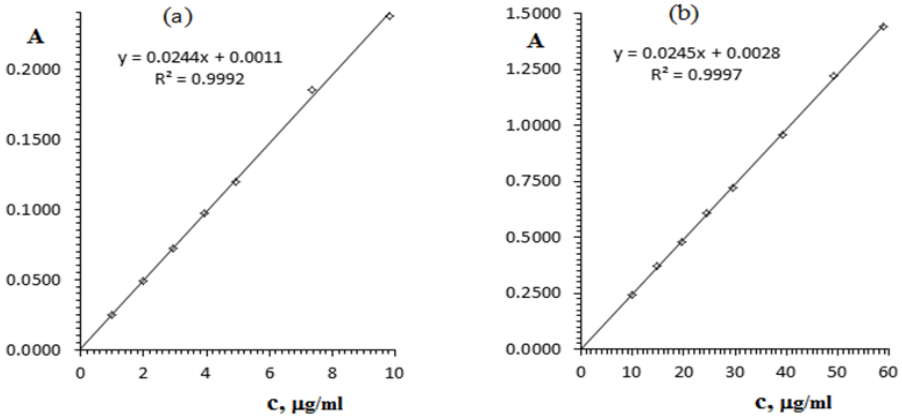


Fig. 5: Calibration curve for determination of GLM according to optimal conditions at λ_{max} 416 nm in present of 1×10^{-4} M of BCG (a) and 1×10^{-3} M of BCG (b); C_{GLM}: 0.981, 1.962, 2.944, 3.925, 4.906, 7.359, 9.812 µg/ml (a) and 9.812, 14.718, 19.625, 24.530, 29.437, 39.249, 49.062 and 58.874 µg/ml (b) {Blank is BCG solution in chloroform: 1x10^{-4}M (a) and 1x10^{-3}M (b); ℓ = 1 cm}

Table 2: The parameters established for spectrophotometric determination of GLM by complex formation with BCG in chloroform

Parameters	Operating values
Molar absorptivity of GLM: BCG complex (ε), l. mol^{-1}. cm^{-1}	1.2x10^4
Regression equation at λ_{max}=416 nm for C_{GLM}= 0.981-9.812 µg/ml:	
Slope	0.0244
Intercept	0.0011
Correlation coefficient (R^2)	0.9992
Regression equation at λ_{max}=416 nm for C_{GLM}= 9.812-58.874 µg/ml:	
Slope	0.0245
Intercept	0.0028
Correlation coefficient (R^2)	0.9997
Beer's Law Limit, for C_{GLM} by µM	2-120
Beer's Law Limit, for C_{GLM} by µg/ml	0.981-58.874
RSD%	3.0
LOD(3.3SD), for C_{GLM} by µg/ml	0.088
LOQ (10SD), for C_{GLM} by µg/ml	0.29

n=5, t=2.776.

Method validation

The developed method for simultaneous estimation of GLM has been validated in accordance with the International Conference on Harmonization guidelines (ICH)[26].

Linearity

Several aliquots of a 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 0.981-58.874 µg/ml for GLM. Absorbance was plotted against the corresponding concentrations to obtain the calibration graph, see fig. 2. Linearity equations obtained were y = 0.0244x+0.0011 for the range 0.891-9.812 µg/ml (R^2=0.9992) and y=0.0245x+0.0028 for the range 9.812-58.874 µg/ml (R^2=0.9997).

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 10µg/ml. 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 98.9% to 102.4%, see table 4.

Table 3: Spectrophotometric determination of GLM through complex formation with BCG within optimal conditions using calibration curve in chloroform

X_i, µg/ml (Taken)	* $\bar{X}$, µg/ml (Found)	SD, µg/ml	$\frac{SD}{\sqrt{n}}$, µg/ml	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$ µg/ml	RSD %	* $\bar{X}$, µg/ml RP-HPLC [25]
0.981	0.98	0.029	0.013	0.98±0.036	3.0	0.98
1.962	1.96	0.058	0.026	1.96±0.072	3.0	1.96
2.944	2.91	0.084	0.038	2.91±0.104	2.9	2.93
3.925	3.93	0.113	0.051	3.93±0.140	2.9	3.93
4.906	4.87	0.136	0.061	4.87±0.169	2.8	4.90
7.359	7.54	0.203	0.091	7.54±0.252	2.7	7.42
9.812	9.71	0.262	0.117	9.71±0.325	2.7	9.80
14.718	14.99	0.389	0.174	14.99±0.483	2.6	14.81
19.625	19.48	0.487	0.218	19.48±0.605	2.5	19.54
24.530	24.78	0.619	0.277	24.78±0.769	2.5	24.62
29.437	29.27	0.731	0.327	29.27±0.908	2.5	29.38
39.248	39.07	0.937	0.419	39.07±1.163	2.4	39.17
49.062	49.68	1.192	0.533	49.68±1.480	2.4	49.41
58.874	57.03	1.311	0.586	57.03±1.628	2.3	57.64

* n=5, t= 2.776

Table 4: Results of recovery studies

Level	% Recovery
80%(n=5)	98.9
100%(n=5)	99.8
120%(n=5)	102.4

Repeatability

The repeatability was evaluated by performing 10 repeat measurements for 9.812 µg/ml of GLM using the studied spectrophotometric method under the optimum conditions. The found amount of GLM ($\bar{x}$ ± SD) was 9.71±0.26 µg/ml and the percentage recovery was found to be 98.98±2.6 with RSD of 0.027. These values indicate that the proposed method has high repeatability for GLM analysis.

Sensitivity (limit of detection [LOD] and limit of quantitation [LOQ])

The sensitivity of the method was evaluated by determining the LOD and LOQ. The values of LOD and LOQ for GLM are 0.088 and 0.26 µg/ml, respectively.

Robustness

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, BCG (±5%), temperature (±5 °C) and reaction time (30 min).

Specificity

The specificity of the method was ascertained by analyzing standard glimepiride in the presence of excipients. There was no interference from most of the common excipients.

APPLICATIONS

The developed spectrophotometric method was applied to determine glimepiride in some pharmaceutical preparations through complex formation by BCG in chloroform according to the optimal conditions. The amount (m) of glimepiride in one tablet was calculated from the following relationship: m = h. m, where: m is the amount of GLM in tablet calculated according to the regression equation (II), h conversion factor is equal to (0.1).

Table 5: Determination of GLM in some Syrian pharmaceutical preparations using spectrophotometric method through complex formation with BCG in chloroform, λ_{max} 416 nm

Tablet dosage form	Label Claim of GLM, mg/tab.	*mean±SD, mg/tab.	RSD %	Assay	* (Assay %), by RP-HPLC [25]
Glimaryl	4	4.096±0.115	2.8	102.4	102.5
	2	1.970±0.060	3.0	98.5	98.7
Amarium	4	4.020±0.113	2.8	100.5	100.3
	2	1.956±0.059	3.0	97.8	98.1

* n=5, Assay=(found mean/label claim)x100.

The results of quantitative analysis for GLM in pharmaceutical preparations were summarized in Tables 5. The proposed method was simple, direct, specific and successfully applied to the determination of GLM in pharmaceuticals without any interference from excipients. Average assay ranged between 97.8 to 102.4%. The results obtained by this method agree well with the contents stated on the labels and were validated by RP-HPLC [25].

CONCLUSION

The developed spectrophotometric method is simple, direct (extraction-free), cost-effective and specific for the determination of glimepiride in pure and tablet dosage forms. This method is based on the formation of ion-pair complex between GLM and bromocresol green in chloroform (λ_{max} 416 nm). Beer's law in the optimum

experimental conditions is valid within a concentration range of 0.981-58.874 µg/ml. The developed method is applied for the determination of GLM in pure and its commercial tablets without any interference from excipients with an average assay of marketed formulations 97.8 to 102.4%.

CONFLICT OF INTERESTS

The authors have declared that no conflict of interests exists.

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