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Spectrophotometric Determination of Glimepiride in Bulk and Tablet Dosage forms Through Formation Ion-Pair Complex Using Metacresol purple.

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Abstract

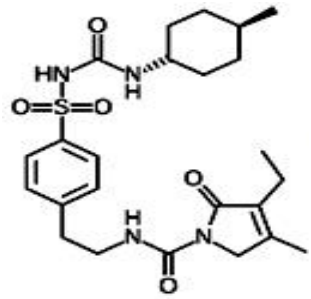
A spectroscopic study of the interaction of glimepiride $C_{24}H_{34}N_4O_5S$ (GLM) with metacresol purple $C_{21}H_{18}O_5S$ (MCP) in chloroform medium and formation of complexes was studied. The method involves the formation of a yellow ion-pair complex between MCP with GLM at pH<3.8; after reacting GLM with Na_2CO_3 to give $C_{24}H_{33}N_4O_5NaS$ which is extracted by chloroform. The formed complex [GLM]:[MCP] was measured at λ_{max} 418 nm against the reagent blank prepared in the same manner. Variables were studied in order to optimize the reaction conditions. Molar absorptivity (ε) for complex was 16800 $L.mol^{-1}.cm^{-1}$. Beer's law was obeyed in the concentration range of 0.4906 - 49.062 $\mu g.mL^{-1}$ in present of $2.0x10^{-4}$ $mol.L^{-1}$ of MCP with good correlation coefficient (R^2= 0.9998). The relative standard deviation did not exceed 3.6%. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.046 and 0.139 $\mu g.mL^{-1}$, respectively. The proposed method was validated for specificity, linearity, precision and accuracy, repeatability, sensitivity (LOD and LOQ), robustness and homogenization of tablets. The developed method is applicable for the determination of GLM in bulk and different dosage forms with average assay of 98.20 to 100.85% and the results are in good agreement with those obtained by the RP-HPLC reference method.

Keywords: Spectrophotometric method, Glimepiride, Metacresol purple, Ion-pair complex

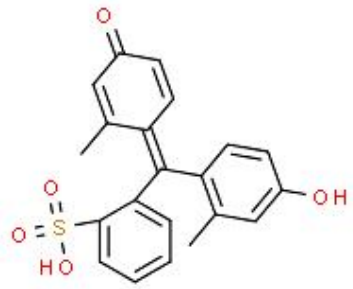
Introduction

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], see scheme1.

Metacresol purple $C_{21}H_{18}O_5S$ (MCP), also called m-cresolsulfonphthalein is a triarylmethane dye and a pH indicator ranges pH 1.2–2.8 appearing red to yellow and pH 7.4–9.0 appearing yellow to purple, mol. mass 382.43 g [2], see scheme 2.



Scheme 1: Chemical structure of Glimepiride



Scheme 2: Chemical structure of Metacresol purple

The ion-associate complexes of diaveridine with metacresol purple were prepared in a solution and studied spectrophotometrically. The bulky counter anions such as sulfonphthalein acidic dyes as MCP were used for ion-associate complexes formation. Most spectrophotometric methods employ extraction procedures [3].

Metacresol purple (MCP) is a triarylmethane dye has been established for the determination of sildenafil in pure and pharmaceuticals. The method is based on the formation of ion-association complex of sildenafil citrate (SC) with metacresol purple (MCP) in acidic buffer solution. The complex can be extracted into chloroform and quantitatively measured at 410 nm [4].

Previous research that identified glimepiride in its pure form and in pharmaceutical preparations was identified according to different methods including spectrophotometry [5-12], high-performance liquid chromatography [13-15], electrochemical [16]. Glimepiride has also been identified spectrally in conjunction with other compounds [17-19].

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 [20]. Also none of the methods reported in the literatures is based on the formation of complex between MCP and GLM.

Experimental

Reagents

Glimepiride (99.98%) was purchased from Chempi Fine Chemicals (INDIA). All reagents (as metacresol purple (99%), chloroform and others) were of analytical grade from Merck.

Instruments and apparatus

Spectrophotometric measurements were made in $T90^{+}$ UV-VIS with 1.0 cm quartz cells. 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). SARTORIUS TE64 (0.01 mg) electronic balance was used for weighing.

A stock standard solution of metacresol purple ($1x10^{-2}$ mol.L^{-1})

Dissolving 96.57 mg of metacresol purple with N,N-dimethylmethanamide into volumetric flask (25 mL) and diluting to mark by N,N-dimethylmethanamide.

A stock standard solution of glimepiride ($1x10^{-3}$ mol.L^{-1})

1×10^{-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 methanol and heating until dry; where the following interaction occurs:

$$C_{24}H_{34}N_4O_5S + Na_2CO_3 \longrightarrow C_{24}H_{33}N_4O_5NaS + HNaCO_3$$

After that it was dissolved in chloroform into a volumetric flask (25 mL) and diluted up to mark with chloroform and mixed well.

Working solutions

The stock solutions were further diluted to obtain working solutions daily just before use in the ranges of GLM: 1.00, 2.50, 5.00, 7.50, 10.00, 20.00, 30.00, 40.00, 50.00, 60.00, 75.00, 85.00, and 100.00 µmol.L^{-1} (0.490, 1.226, 2.453, 3.680, 4.906, 9.812, 14.719, 19.625, 24.531, 29.437, 36.797, 41.703, and 49.062 µg.mL^{-1}) by dilution of the volumes: 10, 25, 50, 75, 100, 200, 300, 400, 500, 600, 750, 850, and 1000 µL from stock standard solutions of glimepiride $1x10^{-3}$ mol.L^{-1} into 10 mL volumetric flask, then added 0.200 mL from stock standard solution of metacresol purple ($1x10^{-2}$ mol.L^{-1}) and diluted to 10 mL with chloroform.

Samples

Commercial formulations (as tablet) were used for the determination of GLM. The pharmaceutical formulations were subjected to the analytical procedures:

(1) ***Amarium*** tab., **RACHA Laboratories**, Aleppo - Syria, each tablet contains: 2 and 4 mg of GLM.
(2) ***Amapiride*** tab., **AVENZOR Pharmaceutical Industries**, Damascus–Syria, each tablet each contains: 2 and 4 mg of GLM.

Stock solutions of 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 methanol 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 contains 2 and 4 mg/tab, respectively.

Working solutions of pharmaceuticals

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 0.200 ml from stock standard solution of MCP 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 they contain $2.0x10^{-4}$ $mol.L^{-1}$ of MCP; test solutions).

Results and Discussion

Analytical procedure

A spectrophotometric method for the determination of glimepiride with metacresol purple has been studied. The method is based on the formation of a yellow ion-pair complex between metacresol purple with glimepiride at pH<3.8; after reacting glimepiride with Na_2CO_3 to give $C_{24}H_{33}N_4O_5NaS$ which is extracted by chloroform. The formed complex [GLM]:[MCP] was measured at λ_{max} 418 nm against the reagent blank prepared in the same manner.

In this study, extraction-free spectrophotometric method for determination of glimepiride through ion-pair complex formation with metacresol purple was developed (for the first time).

Spectrophotometric results

UV-Vis spectra of glimepiride, metacresol purple and the formed complex GLM:MCP solutions in chloroform was obtained. Glimepiride solutions do not absorb in the range 300-600 nm. Metacresol purple solutions have absorption at λ_{max} 404 nm and 532nm ($\varepsilon \approx 950$ $L.mol^{-1}.cm^{-1}$ and 1320 $L.mol^{-1}.cm^{-1}$ in chloroform). [GLM]:[MCP] complex solutions have maximum absorption at λ_{max} 418 nm in chloroform, ε for the complex was 16800 $L.\ mol^{-1}.cm^{-1}$, see Figure 1.

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

The effect of time and temperature

The effect of time and temperature on the complex [GLM]:[MCP] 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.

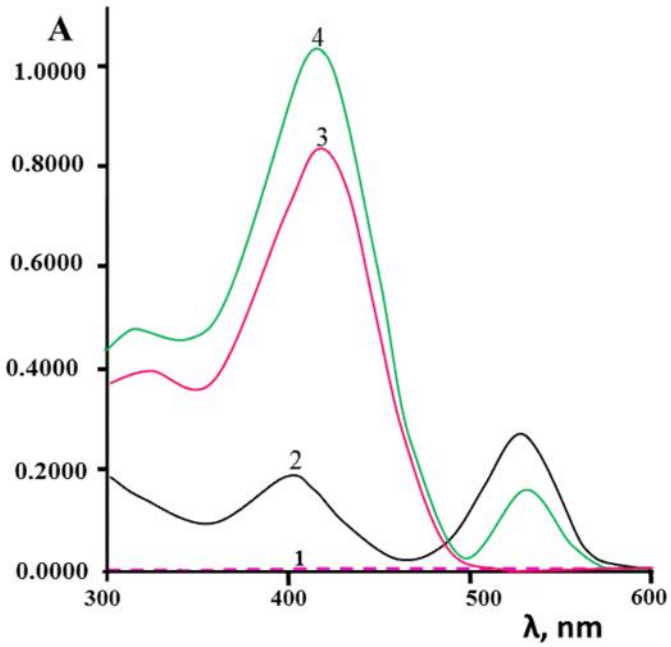


Fig. 1: UV-Vis spectra in is chloroform of: 1- $5x10^{-5}$ mol.L^{-1} of GLM; 2- $2.0x10^{-4}$ mol.L^{-1} of MCP; 3- $5.0x10^{-5}$ mol.L^{-1} ion-pair complex ($5x10^{-5}$ mol.L^{-1} of GLM with $2.0x10^{-4}$ mol.L^{-1} MCP) Blank is $2.0x10^{-4}$ mol.L^{-1} MCP, 4- $5.0x10^{-5}$ mol.L^{-1} ion-pair complex ($5.0x10^{-5}$ mol.$L^{-1}$of GLM with $2.0x10^{-4}$ mol.L^{-1} of MCP); Blank is chloroform, ℓ =1.cm.

The effect of MCP concentration

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

Stoichiometric relationship

The molar ratio method

The composition of GLM:MCP complex were determined by the molar ratio method and Job's method of continuous variation [20]. The stoichiometry of GLM:MCP complex was studied by molar ratio method according to following equation: A_{max}= f ([MCP]/[GLM]) at λ_{max} 418 nm in chloroform. It confirmed that the binding ratio of GLM: MCP complexes are equal to (1:1); where the concentration of GLM was constant (50 µmol.L^{-1}) and the concentrations of MCP changed from 0 to 250 µmol.L^{-1}, see Figure 2. The formation constant of the ion pair complex [GLM]:[MCP] is $1.18x10^{7}$ in chloroform.

The Job's method

Continuous variation was utilized to check the composition of GLM:MCP complex at λ_{max} 418 nm in chloroform. The absorbance of the complex in used solvent were plotted against the mole fraction [MCP]/([GLM]+[MCP), where [GLM]+[MCP]=100 µmol.L^{-1}. The plot reached maximum value at a mole fraction of 0.5, see Figure 3. This indicated complex formation (GLM:MCP) in the ratio of (1:1). The formation constant of the ion- pair complex [GLM]:[MCP] is $1.98x10^{7}$.

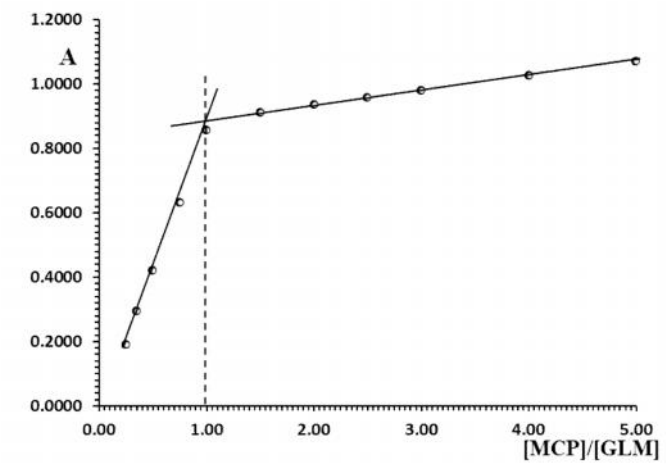


Fig. 2: Molar ratio method to calculate binding ratio of GLM:MCP complex at λ_{max} 418 nm in chloroform ([GLM]= 50 µmol.L^{-1}, blank is chloroform, ℓ =1 cm).

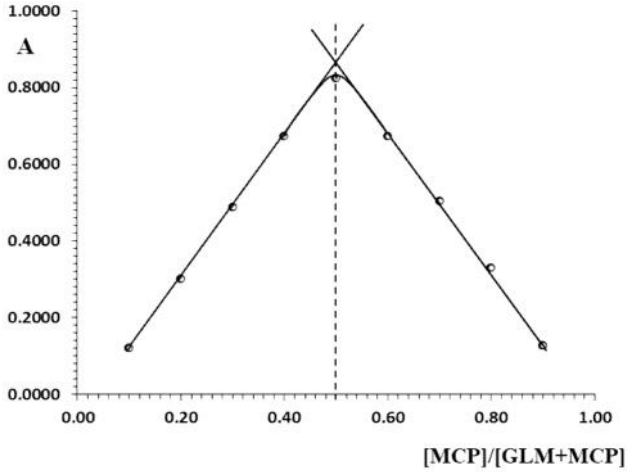
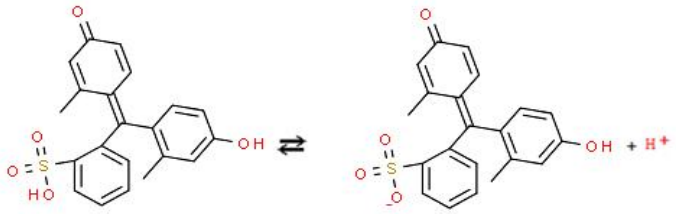


Fig. 3: Job's method of continuous variation to calculate binding ratio of GLM:MCP complex at λ_{max} 418 nm in chloroform ([GLM]+[MCP]=100 µmol.L^{-1}, blank is chloroform, ℓ =1 cm).

Mechanism of Reaction:

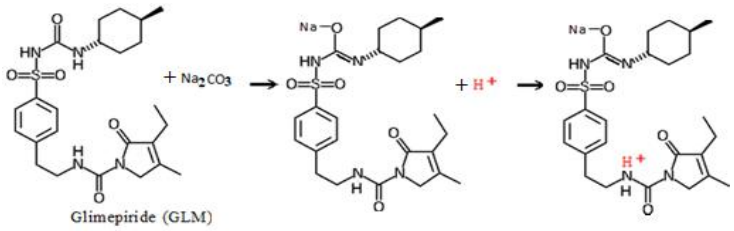
Anionic dyes such as MCP form ion-pair complexes with the positively charged nitrogen-containing molecule. The color of such dyes is due to the opening of lactoid ring and subsequent formation of quinoid group (deprotonated). Glimepiride ($C_{24}H_{34}N_4O_5S$) is reacted with Na_2CO_3 to give ($C_{24}H_{33}N_4HO_5NaS$) and then it interacts with H^+ (from MCP) and forms yellow ion-pair complexes [GLM]:[MCP] with the dye. Drug-dye complex with oppositely charged ions (positive on the drug and negative on the dye) behaves as a single unit held together by an electrostatic binding. The suggested mechanism of GLM:MCP ion-pair complexes formation is shown in Scheme 3.

i- First step:

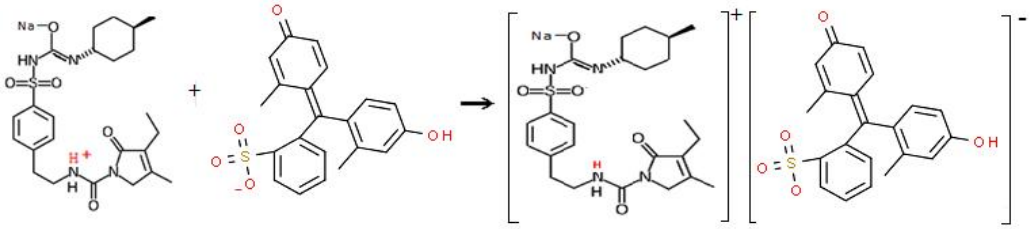


Metacresol purple (MCP)

ii-Second step:



iii- Third step (formation of [GLM]:[MCP]):



[GLM]:[MCP]

Scheme 3: suggested mechanism of [GLM]:[MCP] ion-pair complexes formation.

Calibration curve

The spectra of GLM in pure form through complexation with MCP showed excellent linearity over concentration range of 0.490-49.062 $\mu g.mL^{-1}$ (1.00-100.00 $\mu mol.L^{-1}$) in presence of 2.0×10^{-4} $mol.L^{-1}$ of MCP was studied, see Figure 4. The calibration curve of complex [GLM]:[MCP] showed in Figure 5. Regression equation at λ_{max} was as the follows: y=0.0342x +0.0013 with good correlation coefficient (R^2= 0.9998) in chloroform. The spectra characteristics of the method such as the molar absorptivity (ε, Beer's law, regression equation at λ_{max} (y=a.x+b); where y=absorbance, a=slope, x=concentration of GLM by $\mu g.mL^{-1}$, b=intercept, the correlation coefficient, limit of detection (LOD), limit of quantification (LOQ) and the optimum conditions for spectrophotometric determination of GLM through ion-pair complex formation using MCP in chloroform were summarized in Table 1.

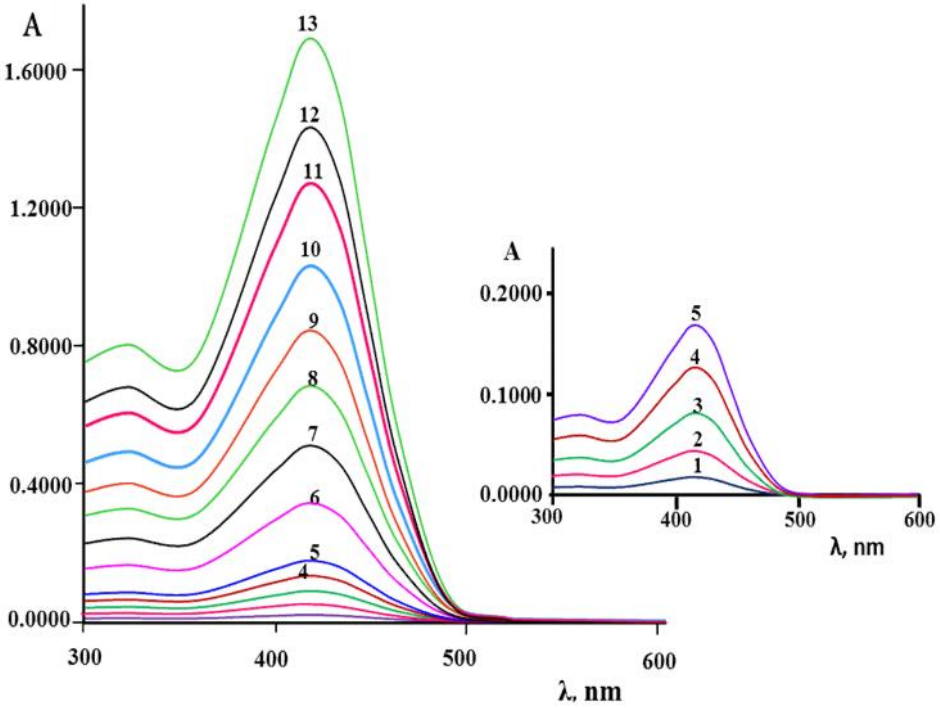


Fig. 4: Spectra of [GLM]:[MCP] complex in presence of 2.0×10^{-4} mol.L^{-1} of MCP; where C_{GLM} as the follows: 1- 0.490, 2- 1.226, 3- 2.453, 4- 3.680, 5- 4.906, 6- 9.812, 7- 14.719, 8- 19.625, 9- 24.531, 10- 29.437, 11- 36.797, 12- 41.703 and 13- 49.062 µg.mL^{-1}

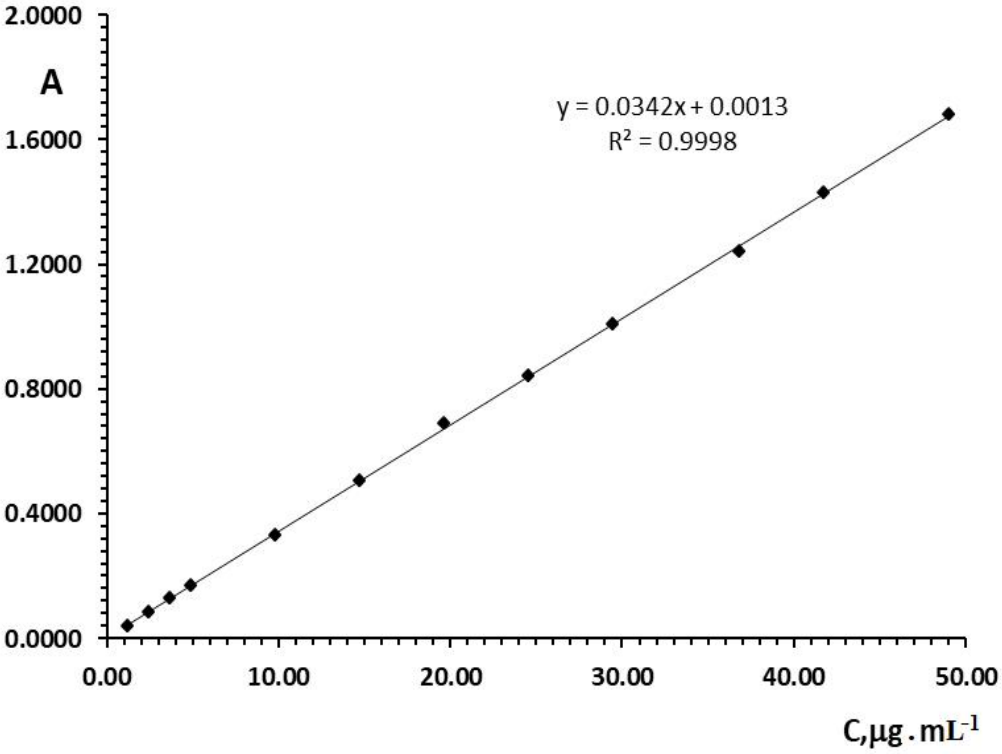


Fig. 5: Calibration curve for determination of GLM according to optimal conditions at λ_{max} 418 nm (in presence of 2.0×10^{-4} mol.L^{-1} of MCP) where GLM: 0.490 - 49.062 µg.mL^{-1} {Blank is MCP solution in chloroform 2.0×10^{-4} mol.L^{-1} ; ℓ = 1 cm}.

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

Parameters	Operating values
λ_{max} of GLM:MCP complex, nm	418
Beer's Law Limit, for C_{GLM} by $\mu mol.L^{-1}$	1-100
Beer's Law Limit, for C_{GLM} by $\mu g.mL^{-1}$	0.490 - 49.062
Molar absorptivity of [GLM]:[MCP] complex (ε), $L.mol^{-1}.cm^{-1}$	16800
Regression equation for [GLM]:[MCP] at λ_{max} 418 nm:	
Slope	0.0342
Intercept	0.0013
Correlation coefficient (R^2)	0.9998
LOD for C_{GLM} by $\mu g.mL^{-1}$ in [GLM]:[MCP]	0.046
LOQ for C_{GLM} by $\mu g.mL^{-1}$	0.139
RSD%	3.6
C_{MCP}:C_{GLM}, M	≥2
Stability	10 h
Temperature of solution	20±5°C

n=5, t=2.776.

Analytical results determination of Glimepiride

Spectrophotometric determination of GLM through complexation with MCP 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 0.490 to 49.062 $\mu g.mL^{-1}$, 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 [13] and good agreement was observed between them.

Table 2: Spectrophotometric determination of GLM through complex formation with MCP within optimal conditions using calibration curve in chloroform (*n=5, t=2.776).

X_i, (Taken)		*$\bar{x}$ ±SD, $\mu g.mL^{-1}$ (Found)	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$ $\mu g.mL^{-1}$	RSD%	*$\bar{x}$,$\mu g.mL^{-1}$ RP-HPLC[13]
$\mu mol.L^{-1}$ (μM)	$\mu g.mL^{-1}$				
1.00	0.490	0.488±0.018	0.488±0.022	3.6	0.485
2.50	1.226	1.227±0.043	1.227±0.053	3.5	1.218
5.00	2.453	2.459±0.084	2.459±0.104	3.4	2.461
7.50	3.680	3.700±0.122	3.700±0.152	3.3	3.707
10.00	4.906	4.882±0.151	4.882±0.188	3.1	4.900
20.00	9.812	9.810±0.304	9.810±0.377	3.1	9.865
30.00	14.719	14.904±0.447	14.904±0.555	3.0	14.912
40.00	19.625	19.682±0.590	19.682±0.733	3.0	19.724
50.00	24.531	24.522±0.711	24.522±0.883	2.9	24.621
60.00	29.437	29.440±0.824	29.440±1.023	2.8	29.543
75.00	36.797	36.527±0.986	36.527±1.224	2.7	36.800
85.00	41.703	41.804±1.087	41.804±1.349	2.6	41.714
100.00	49.062	49.103±1.228	49.103±1.524	2.5	49.103

Applications

The developed spectrophotometric method was applied to determine GLM in some Syrian pharmaceutical preparations through complex formation by MCP 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. The assay ranged between 98.20 to 100.85%. The results obtained by this method agree well with the contents stated on the labels and were validated by RP-HPLC method [13].

Table 3: Determination of GLM, in some Syrian pharmaceutical preparations using spectrophotometric method through complex formation with MCP in chloroform, λ_{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[13]	* Assay %, by RP-HPLC[13]
Amarium	2	2.017±0.063	3.1	100.85	2.005±0.033	100.25
	4	3.992±0.112	2.8	99.60	3.987±0.054	99.68
Amapiride	2	1.964±0.061	3.1	98.20	1.990±0.034	99.50
	4	3.935±0.110	2.8	98.38	3.940±0.055	98.50

***** n=5.

Method validation

The developed method for simultaneous estimation of GLM has been validated in accordance with the International Conference on Harmonization guidelines (ICH) [21].

Selectivity

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.

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 0.490 - 49.062 $\mu g.mL^{-1}$ for GLM. Absorbance was plotted against the corresponding concentrations to obtain the calibration graph, see Figures 4 & 5 and Table 2. Linearity equations obtained were $y = 0.0342x + 0.0013$ for the range 0.490 - 49.062 $\mu g.mL^{-1}$ (R^2=0.9998).

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 from test solution of pharmaceuticals was contented 20.00 $\mu g.mL^{-1}$. 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 100.2% to 102.1%, see Table 4.

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

Level	% Recovery
80%	100.5
100%	100.2
120%	102.1

Repeatability

The repeatability was evaluated by performing 10 repeat measurements for 19.625 µg.mL^{-1} of GLM using the studied spectrophotometric method under the optimum conditions. The found amount of GLM ($\bar{x}$ ± SD) 19.682 ± 0.513 µg.mL^{-1} and the percentage recovery was found to be 100.29 ± 2.6 with RSD of 0.026. These values indicate that the proposed method has high repeatability for GLM analysis.

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 0.046 and 0.139 µg.mL^{-1}, 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, λ_{max} of GLM:MCP complex (418±1nm), temperature (20±5^{o}C), stability (10±5% h) and reaction time (5±1 min), see Table 5. 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}	29.437 µg.mL^{-1}
Temperature		
15^{o}C	99.4	99.7
25^{o}C	100.1	101.1
Stability		
9.5 h	100.0	100.1
10.5 h	100.2	100.3
Reaction time		
4.0 min	99.7	99.9
6.0 min	100.0	100.1
λ_{max} , nm		
417	99.8	99.8
419	99.9	99.9

* n=5.

The homogenization of tablets

The homogenization of tablets in terms of the weight and the amount of drug was studied. It found that the mean weight and amount drug in the tablets was 0.1795 ± 0.0031 g (i.e. ±1.73%), 0.1794 ± 0.0024 g (i.e. ±1.34%) for Amarium tablets (2 and 4 mg/tab) and 0.1003 ± 0.0015 g (i.e. ±1.50%) and 0.0995 ± 0.0013 g (i.e. ±1.31%) Amapiride tablets (2 and 4 mg/tab), respectively. While the mean amount drug in the tablets was 1.997±0.071 mg/tab (i.e. ±3.6%) and3.983±0.120 mg/tab (i.e. ±3.0%) for Amarium tablets (2 and 4 mg/tab) and 2.002±0.074 mg/tab (i.e. ±3.7%) and

3.976±0.124 mg/tab (i.e. ±3.1%) for Amapiride tablets (2 and 4 mg/tab), respectively; which shows that homogeneity of tablets is good.

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 MCP in chloroform ([GLM]:[MCP]. Beer's law in the optimum experimental conditions using [GLM]:[MCP] complex is valid within a concentration range of 0.490 - 49.062 $\mu g.mL^{-1}$. 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 98.20 to 100.85%.

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