Agriculture College – Wasit University

Dijlah Journal of Agricultural Sciences

ISSN 2790 – 5985
eISSN 2790 – 5993

Dijlah J. Agric. Sci.، 4(2):79-95, 2025

# Development of an Analytical Method for Ligand Quantification in Pharmaceutical Forms Using Visible Range Molecular Absorption Spectroscopy

**Nawras Majid Hassouni**

**Ministry of Education / Wasit Education Directorate, AL-Kut, Iraq.**
Corresponding author: nawrasmajidhassouni@gmail.com

**Abstract**:

The research includes for the assessment of ligands as raw materials and in pharmaceutical preparations, the research entails the development of a sensitive, cost-effective, and specific spectroscopic analytical approach. The suggested technique uses a nucleophilic substitution mechanism to create an orange-red complex that exhibits distinctive absorbance at 450 nm. The optimal experimental conditions for the reaction were studied, with the best conditions being the use of 1.4 ml of reagent at a concentration of 2.2% w/v, in the presence of methanol as a solvent, and heating to 90°C in a water bath for 22 minutes. The stability of the product and the ratio of the reactants involved in its formation were also studied, according to the Job method and the molar ratio method. The results showed that the resulting complex was stable for up to two hours, and that the reaction ratio was one to one for each reagent. The analytical method was evaluated according to ICH rules under optimum reaction conditions. Lambert-Beer's law was applied within concentrations (30-110 M) with a correlation coefficient of R=0.99 and recovery values ranging from 99.39-99.59%. The method was successfully used to determine the ligand in tablets, as no obstruction was observed from the excipients included in the composition of these preparations or metformin in the participating pharmaceutical forms. It was found that there were no statistically significant differences when comparing the results with the results of the assay by HPLC and UV-vis absorption methods in terms of t-test and F-test. The finding results of the docked compound Gliclazide with binding affinity – 7.7 kcal/mol, the results show us the compound's ability to inhibit the enzyme UNC, which promises to inhibit and treat inflammation in the udder of cows, thus achieving the highest possible levels of milk production.

**Keywords***: Ligand, Dosage Forms, Visible Spectrophotometer.*
**Received:1/5/2025** **Accepted:13/7/2025** **Published:30/7/2025**

## تطوير طريقة تحليلية لتقدير كمية اللِّيجاند في الأشكال الصيدلانية باستخدام مطيافية الامتصاص الجزيئي في الطيف المرئي

**نورس ماجد حسوني**

**وزارة التربية / مديرية تربية واسط، الكوت، العراق.**

**الخلاصة**

يتناول البحث تقدير الليجاندات سواء كمواد أولية أو في المستحضرات الصيدلانية، حيث يتضمن تطوير طريقة تحليلية طيفية حساسة وفعّالة من حيث التكلفة وذات خصوصية عالية. تعتمد التقنية المقترحة على آلية الإحلال النوكليوفيلي لتكوين معقد برتقالي-أحمر يظهر امتصاصًا مميزًا عند طول موجي 450 نانومتر. تم دراسة الظروف التجريبية المثلى للتفاعل، حيث وُجد أن أفضل الظروف تتحقق باستخدام 1.4 مل من الكاشف بتركيز 2.2% وزن/حجم، مع استخدام الميثانول كمذيب، والتسخين في حمام مائي عند 90°م لمدة 22 دقيقة. كما تمت دراسة ثباتية الناتج ونسبة المتفاعلات المشاركة في تكوينه باستخدام طريقة "جوب" وطريقة النسبة المولارية. أظهرت النتائج أن المعقد الناتج مستقر لمدة تصل إلى ساعتين، وأن نسبة التفاعل هي (1:1) بين الكاشف والليجاند. جرى تقييم الطريقة التحليلية وفقًا لمتطلبات ICH تحت ظروف التفاعل المثلى. وقد تم تطبيق قانون لامبرت-بير ضمن مدى تراكيز (30–110 ميكرومول) مع معامل ارتباط R=0.99 وقيم استرجاع تراوحت بين 99.39–99.59%. استُخدمت الطريقة بنجاح في تقدير الليجاند في الأقراص الدوائية، حيث لم يلاحظ أي تداخل من المواد المضافة الداخلة في تركيب هذه المستحضرات أو من عقار الميتفورمين الموجود في بعض الأشكال الصيدلانية المشاركة. كما تبين عدم وجود فروق ذات دلالة إحصائية عند مقارنة النتائج مع نتائج الفحص باستخدام طريقتي HPLC و UV-Vis من حيث اختبار t واختبار .F وأظهرت نتائج الارتباط الجزيئي للمركب Gliclazide بطاقة ارتباط –7.7 كيلوكالوري/مول، مما يدل على قدرة المركب على تثبيط إنزيمUNC ، وهو ما يعد واعدًا في تثبيط وعلاج التهابات ضرع الأبقار، وبالتالي المساهمة في تحقيق أعلى مستويات ممكنة من إنتاج الحليب.

الكلمات المفتاحية : *ليجاند، الأشكال الصيدلانية، مطياف الامتصاص المرئي.*

## 1. INTRODUCTION

UV-VIS spectroscopy is considered as the most important spectrophotometric technique that is most widely used for the analysis of variety of compounds. This technique works on the basis of the measurement of interaction of electromagnetic radiations (EMR) with matter at particular wavelength (28). Spectroscopy in the visible and ultraviolet ranges of the electromagnetic spectrum is one of the most important analytical methods widely used in various fields of analytical chemistry, including clinical, environmental, and pharmaceutical. Its importance in drug monitoring is growing due to its ease, speed, and high sensitivity it is used to identify substances at concentrations different (1)

In addition to its low cost compared to other physicochemical analysis methods, this has made it a focus of interest for researchers working in this field, who are constantly working to develop it. This is done either by improving the design of spectroscopic instruments or the computer programs they are equipped with, or by creating new spectroscopic methods using chemical derivatization techniques. This can help solve some of the analytical problems that compounds suffer from, such as the lack or absence of chromophores, and the occurrence of excipient interference in the compound's spectrum in the ultraviolet range (2) . This can increase the distinctness of the spectrum by forming a measurable colored derivative in the visible range.

The oral hypoglycemic medication Gliclazide, a second-generation sulphonylurea, is used to treat non-insulin-dependent diabetic mellitus (NIDDM). It may help patients with NIDDM regain their insulin resistance and improve impaired insulin secretion. A decrease in blood glucose levels that is sustained throughout the course of both short-term and long-term dosing

and is equivalent to that attained by other sulphonylurea medications is indicative of these actions. Gliclazide's hemobiological effects may make it helpful for diabetic retinopathy patients, according to gradually mounting data, and gliclazide's inclusion to insulin therapy allows for a reduction in insulin dosage.

Therefore, gliclazide is a useful medication for treating the metabolic abnormalities linked to NIDDM and may also have the benefit of reducing the development of diabetic retinopathy. Gliclazide's low incidence of hypoglycemia and good overall tolerability have made it a suitable addition to the range of oral hypoglycemic medications for the treatment of non-insulin-dependent diabetic mellitus (29).

Target Protein of Interest N-acetylglucosamine-1-phosphate uridyltransferase (GlmU) is a key bifunctional enzyme involved in the biosynthesis of UDP-N-acetylglucosamine (UDP GlcNAc), a vital precursor in the formation of peptidoglycan, an essential component of bacterial cell walls. The enzyme possesses both acetyltransferase and uridyltransferase activities, enabling it to catalyze sequential steps in the biosynthetic pathway. Due to its essential role in bacterial survival and the lack of a human homolog with identical functions, GlmU has been recognized as a potential antibacterial drug target.

Based on chemical derivatization, this research developed a new colorimetric spectrophotometric analytical method for measuring gliclazide as a starting material. The optimal conditions for the derivatization reaction were determined, while the selectivity of the method and the accuracy and validity of the results were verified. The study also investigated the feasibility of its application to pharmaceutical preparations, ensuring that these preparations contain gliclazide in accordance with the requirements of pharmacopoeias. It was also compared to other analytical methods.

## Molecular absorption spectroscopy in the visible and ultraviolet range:

When a beam of electromagnetic radiation passes through a material, a number of processes can occur, including scattering, reflection, absorption, and emission. However, in UV-VIS spectroscopy, we only want absorption to occur where the light energy is sufficient to cause electrons to transition from lower, stable energy levels (π and σ) to higher, excited states (i.e., from bonding orbitals containing free electrons (n) to anti-bonding orbitals (π* and σ*), which are in excited states. The nature of the mobility varies according to the energy of the absorbed light rays. The mobility of unsaturated bonds (ππ* saturated bonds) requires more energy than the mobility of (σσ* *nσ, *nπ) and occurs in molecules containing free electrons, i.e. those containing N, O, S, halogen atoms (whether saturated or unsaturated (3) . Figure (1 ) shows the electronic mobility and the energy required for each of these mobility (4).

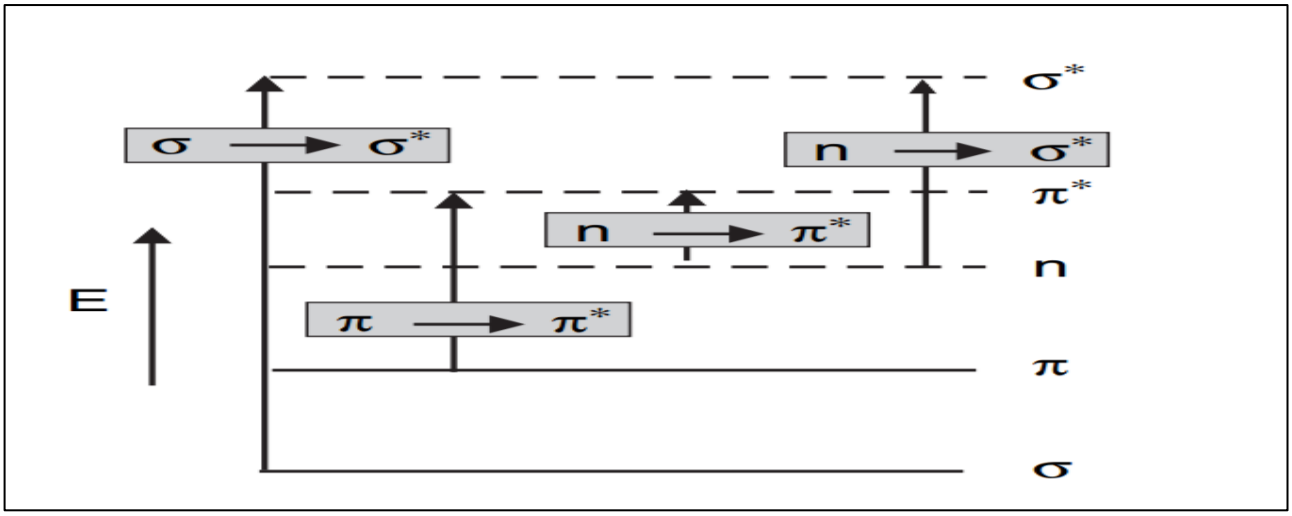


**Figure 1:** Electronic movements and the energy required for them

The functional groups responsible for absorption in the visible and ultraviolet range are called chromophores. The Lambert-Beer equation and absorbance measurements at the wavelength of maximum absorption can be used to calculate a sample's concentration in a solution. (5). Quantitative measurement is not limited to compounds that have an absorption spectrum in that spectral region, but rather includes all compounds that undergo modification by reagents to form measurable derivatives.

## Chemical derivatization

It is an indirect spectroscopic method that can be applied to compounds either due to a lack or absence of chromogens, making them incompatible with UV-VIS spectroscopy, or due to interference between the spectrum of the compound and the spectrum of other materials, such as solutions, excipients, biological materials, and other pharmaceuticals, especially within the UV range. This modification involves adding a suitable reagent to form a colored complex, shifting the wavelength of maximum absorption to the UV-VIS range, thus increasing the sensitivity and selectivity of the analytical method (6).

## The solvent used:

The polarity of the solvent plays a role in the absorption spectrum, as the position and intensity of the absorption band vary depending on the nature of the solvent used. The interference between the solvent and the dissolved substance affects the energy required for the electron transition to occur. The greater the polarity of the solvent, the greater the shift of the maximum absorption wavelength toward shorter wavelengths in the nπ* mobility state, and the shift of the wavelength toward longer wavelengths in the ππ* mobility state (7).Among the most important conditions that must be met by solvents used in spectroscopic analysis are:

- It must not interact with the sample to be measured.

- It must not exhibit absorption at the measurement wavelength.

- It must be a good solvent for the sample and detector.

- It must be highly pure, given the effect of impurities on absorption.

- Water is the best solvent, and organic solvents are used for organic molecules (8).

## Among the pharmaceutical applications used in the quantitative analysis of pharmaceutical substances using this mechanism

The derivation of cephalosporins (Ceftriaxone, Ceftazidime, Cefixime, Cefotaxime, Cefuroxime) containing a primary aromatic amine by the dialysis mechanism, followed by the formation of a colored azo dye with DMAB, where its maxλ ranged from 432 nm to 422 (9). Figure (2) illustrates the reaction mechanism of both ceftriaxone and cefixime.

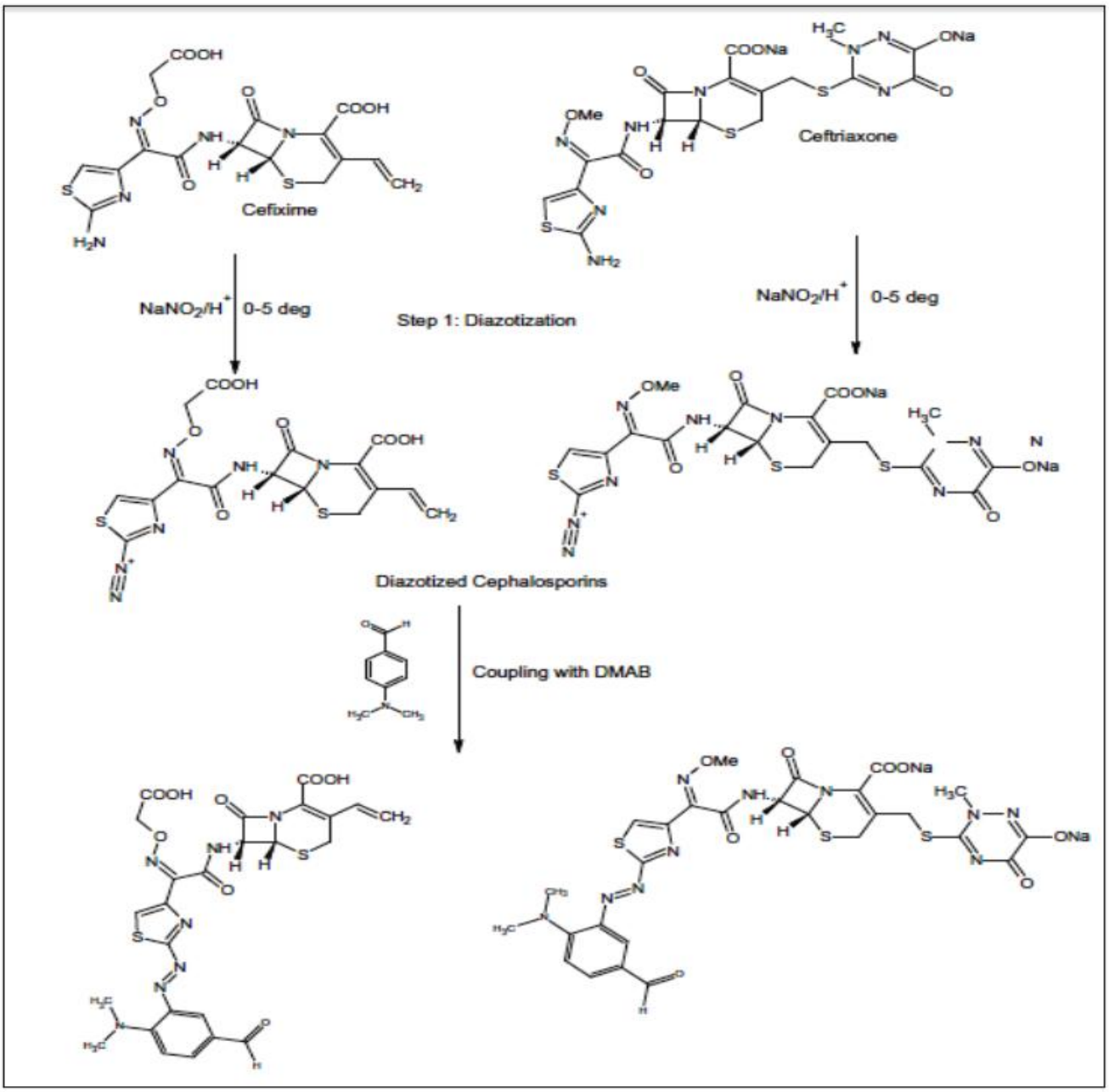


**Figure (2):** Mechanism of reaction of Ceftriaxone, Cefixime with DMAB to form azo dye

This study amid to identify the Gliclazide as potaintial inhibitor against Bovine Mastitis using the enzyim N-acetylglucosamine 1-phosphate uridyltransferase (GlmU), using Molecular docking Method. As the last final step of this method the simulation of molecular docking using Aotu Dock4 for 7 hrs to specify the pocket active site interaction

## 2. MATERIALS AND METHODS

### 2.1 Development of an analytical method for the assay of gliclazide using a reagent

a-Materials and reagents

**Standard materials:**

**-Gliclazide Standard, produced by Jiuzhou - China, with a purity of 99.25%.**

-Metformin hydrochloride with a purity of 99.25%.

## Procedure

A series of standard of solutions with concentrations ranging from 30 to 110 µg/ml was prepared by taking the necessary volumes of the 1000 µg/ml gliclazide standard solution using a micropipette. 1.5 ml of Naphtoquinone-4-sulfonic acide sodium salt( NQS) reagent solution was added to each solution. After sealing the solutions, they were mixed and transferred to a water bath at 90°C. They were then heated for 20 minutes under constant pressure. The serial dilutions were then cooled and the volume was topped up with methanol to 12 ml using a calibrated balloon.

The optical absorbance of the resulting solutions was measured using a spectrophotometer after determining the wavelength of maximum absorption. Table (1) shows the method for preparing the standard series.

**Table (1):** Preparation of the standard series in the gliclazide assay using the developed derivatization mechanism

| Solution No. | 1 | 2 | 3 | 4 | 5 | 6 | Blank |
|---|---|---|---|---|---|---|---|
| Volume of gliclazide solution Standard 1000 mcg/ml (ml) | 0.3 | 0.4 | 0.6 | 0.8 | 1 | 1.1 | - |
| Volume of methanol (ml) | 1.7 | 1.6 | 1.4 | 1.2 | 1 | 0.9 | 2 |
| NQS 0.2% reagent volume (ml) | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Volume of methanol (ml)After heating, add sufficient volume up to 12 ml. | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Corresponding concentration (mcg/ml) (ppm) | 30 | 40 | 60 | 80 | 100 | 110 | - |

## 2.2 Determine the wavelength of maximum absorption:

Select one of the solutions in the series (solution No. 4 with a concentration of 80 µg/ml) and then perform a spectral scan of this solution in the range (400-800 nm).

## 2.3 Setting the derivatization conditions:

### 2.3.1. Effect of the solvent:

Some solvents (methanol, water, acetonitrile) were tested for both gliclazide 50 µg/ml and the Naphtoquinone-4-sulfonic acide sodium salt (NQS) reagent. Chemical derivatization was then performed and the corresponding absorbance was read after determining the maximum absorption wavelength of the resulting derivatization compound in each case, as shown in the table (2).

| The storage used for gliclazide | The storage used for the detector | The storage used for extension |
|---|---|---|
| Methanol | Water | Methanol |
| Methanol | Water | Water |
| Methanol | Methanol | Methanol |
| Acetonitrile | Water | Acetonitrile |
| Acetonitrile | Methanol | Water |

### 2.3.2. Effect of reagent concentration:

Increasing concentrations of the Naphtoquinone-4-sulfonic acide sodium salt (NQS) reagent was tested using methanol as the solvent, starting from 0.08 g/100 ml to 0.5 g/100 ml, with the gliclazide concentration fixed at 50 µg/ml. Chemical derivatization was then applied, and the corresponding absorbance was read at a wavelength of 454 nm.

### 2.3.3. Effect of reagent volume:

Increasing volumes of 0.2% w/v Naphtoquinone-4-sulfonic acide sodium salt (NQS) reagent was tested using methanol as the solvent, starting from 0.5 ml to 3 ml, with the gliclazide concentration fixed at 50 µg/ml. Chemical derivatization was then applied, and the corresponding absorbance was read at a wavelength of 454 nm.

### 2.3.4. Effect of temperature:

To determine the effect of temperature on the chemical derivatization of gliclazide, various temperatures (room temperature 25 - 60 - 70 - 80 - 90) °C were tested in a water bath for 15 - 60 minutes, with the gliclazide concentration fixed at 50 mcg/ml, and the absorbance was read at a wavelength of 454 nm.

### 2.3.5. Effect of heating time:

The effect of heating time on chemical derivatization was studied by heating the sample solution to 90°C in a water bath for increasing time periods (10, 15, 20, 25, 30, and 60 minutes) while maintaining a constant gliclazide concentration of 50 µg/ml and reading the corresponding absorbance at a wavelength of 454 nm.

### Standard addition method:

This is one of the approved methods for measuring the active ingredient in its pharmaceutical form. Each time, a specific amount of the active ingredient in its pharmaceutical form is mixed with a standard amount equal to 80-100-120% of the active ingredient. Chemical derivatization is then applied, and the corresponding absorbance is read (10). The concentration is obtained by substituting it into the straight-line equation obtained from the standard series curve. The results are expressed by calculating the percentage recovery.

### Method:

Twenty tablets were accurately weighed, and the average weight per tablet was calculated. The tablets were then crushed. A 12.5 mg equivalent of the active ingredient was taken and mixed until homogeneous with 10, 12.5, and 15 mg of standard gliclazide, respectively. The mixture was placed in a 25 ml calibrated balloon and dissolved in methanol, stirring well. The mixture was then filtered, and the first part of the filtrate was discarded. 5 ml of each of the previous filtrates was taken and diluted to 10 ml using a calibrated balloon. Concentrations of 450, 500, and 550 µg/ml were obtained. The derivation conditions described in paragraph 4.1 were then applied, after taking 1.2 ml of each of the previous solutions using a micropipette. The procedure was repeated three times for each concentration. The optical absorbance was then measured and substituting into the equation obtained from the standard series curve to obtain the practical concentration and calculate the percentage recovery.

### Gliclazide assay in pharmaceutical preparations:

To verify the effectiveness and accuracy of the developed method for derivatizing gliclazide using the Naphtoquinone-4-sulfonic acide sodium salt (NQS) reagent, the quantitative determination of gliclazide was performed in several local and foreign pharmaceutical forms. The results were compared with those obtained using the standard method based on HPLC and UV-vis absorption spectrophotometry.

### 2.4. Protein Selection and preparation

We obtained the enzyim N-acetylglucosamine 1-phosphate uridyltransferase (GlmU) 3D structure from the RCSB Protein Data Bank (PDB ID: 1FWY). Water molecules in the protein structure were eliminated using Pymol program to produce protein structure resolution 1.50Å (Schrödinger, 2021). Kollman and Gasteiger charges were applied to the

protein structure's amino acid residues after polar hydrogen atoms were added using the AutoDockTools4 program (Morris et al., 2009).

## 2.5. Ligand Structure Preparation

## Certainly. Here's your original sentence, written exactly as you requested:

We obtained the 2D ligand coordinates of Gliclazide from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/compound/3475). O'Boyle et al. (2011) used OpenBabel 3.1.1 software to convert all the ligands to a 3D SDF file.

## 2.6. Molecular Docking

The candidate drug was molecularly docked using Pyrex software in conjunction with AutoDock 4.2 and Autodock Vina, employing the limited cocrystalline binding site as a chemical search space.
(Dallakyan & Olson, 2015). For ligand docked inside the Grid Box, the scoring function was developed using the Lamarckian genetic process. This work employed AutoDock 4.2 for molecular docking.

# RESULTS AND DISCUSSION

## 3.1. Development of an analytical method for the assay of gliclazide.

## Determination of the maximum absorption wavelength:

After conducting a spectral scan of the 82 µg/ml gliclazide solution derived with the 1,2-Naphtoquinone-4-sulfonic acide sodium salt (NQS) detector, it was found that its maximum absorption was at a wavelength of 444 nm, as shown in the figure (3).

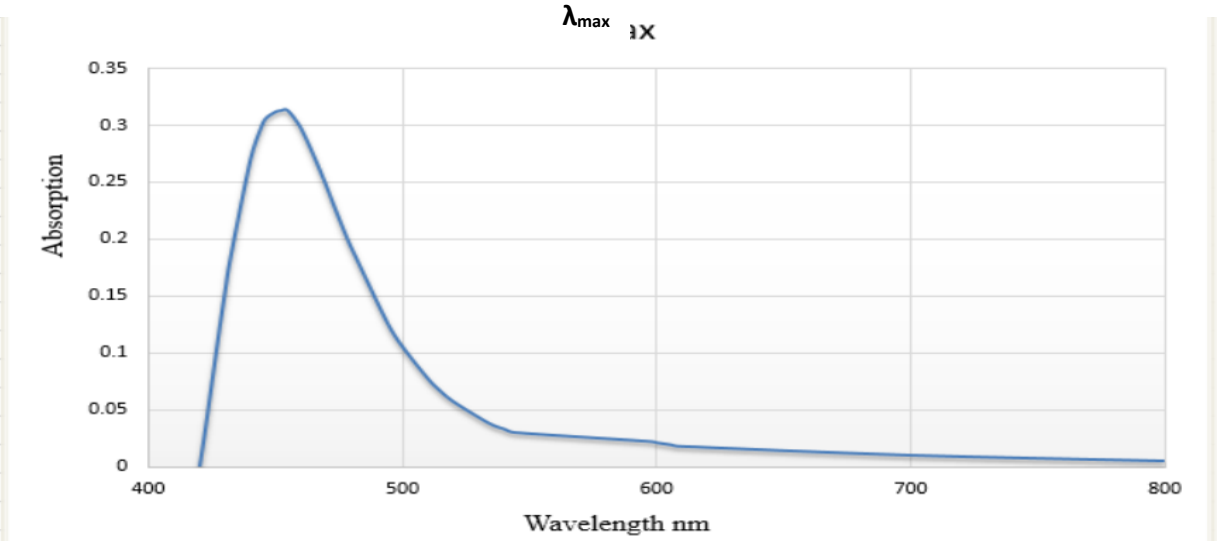


**Figure (3):** Maximum absorption wavelength of the compound resulting from the chemical derivatization of gliclazide 81 µg/ml) using the NQS detector.

### 3.2. Setting the derivatization conditions:

#### 3.2.1. Effect of the solvent:

Table (3) shows the change in the maximum absorption wavelength value of the complex resulting from the derivatization, depending on the solvent used for both the Naphtoquinone-4-sulfonic acide sodium salt (NQS) reagent and gliclazide, and also depending on the solvent used for extension. Methanol showed the highest light absorption value compared to the other solutions.

**Table (3):** Effect of different solvents on the light absorption value of the complex resulting from derivatization (gliclazide concentration 11 µg/ml)

| The place used for gliclazide | The store used for the detector | The store used for extension | $\lambda_{max}$ | Light absorption value |
|---|---|---|---|---|
| Methanol | Water | Methanol | 418 | 2.191 |
| Methanol | Water | Water | 484 | 2.189 |
| Methanol | Methanol | Methanol | 454 | 2.221 |
| Acetonitrile | Methanol | Acetonitrile | 412 | 2.112 |
| Acetonitrile | Methanol | Water | 411 | 2.219 |

#### 3.2.2. Effect of reagent concentration:

Figure (4) shows the effect of using different reagent concentrations (2.282.3 - 2.2 - 2.1 -2.4) g/122 ml on the optical absorption value of the derived complex, where -2.4- concentration 2.2 g/122 ml showed the highest absorption value.

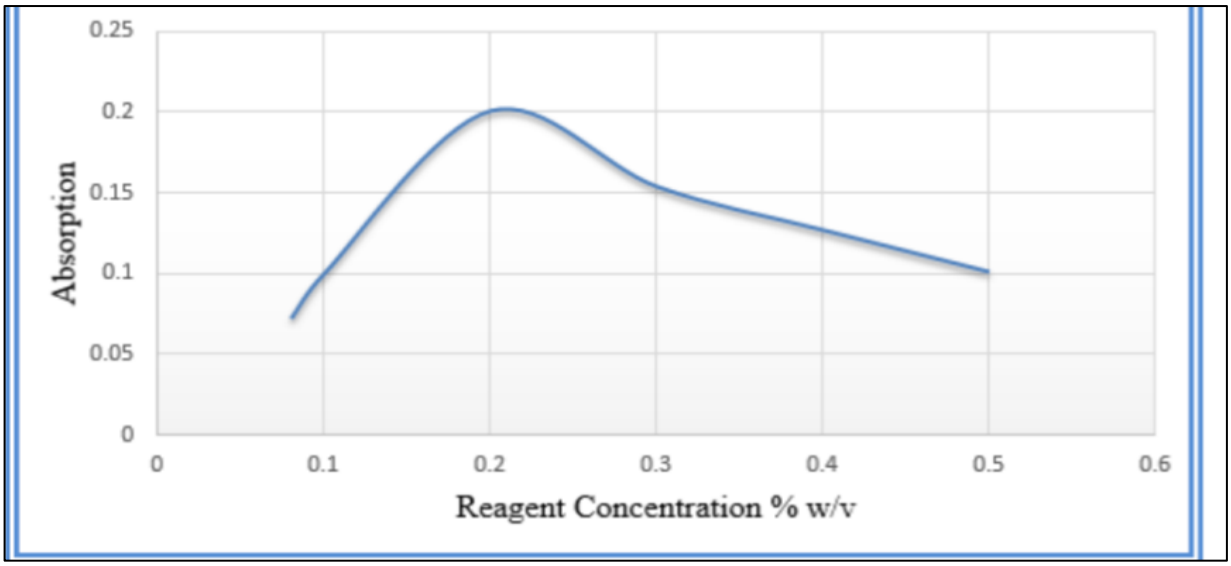


**Figure 4:** Effect of NQS reagent concentration on the absorption value of the resulting complex (gliclazide 11 µg/ml)

### 3.2.3. Effect of reagent volume:

Figure (5) shows the effect of using increasing volumes of Naphtoquinone-4-sulfonic acide sodium salt (NQS) reagent -1 - 2.2% w/v (2.43) ml on the absorbance value of the derived complex, where the absorbance value was -2.4 -2-1.4 maximum using a volume of 1.4 ml of reagent.

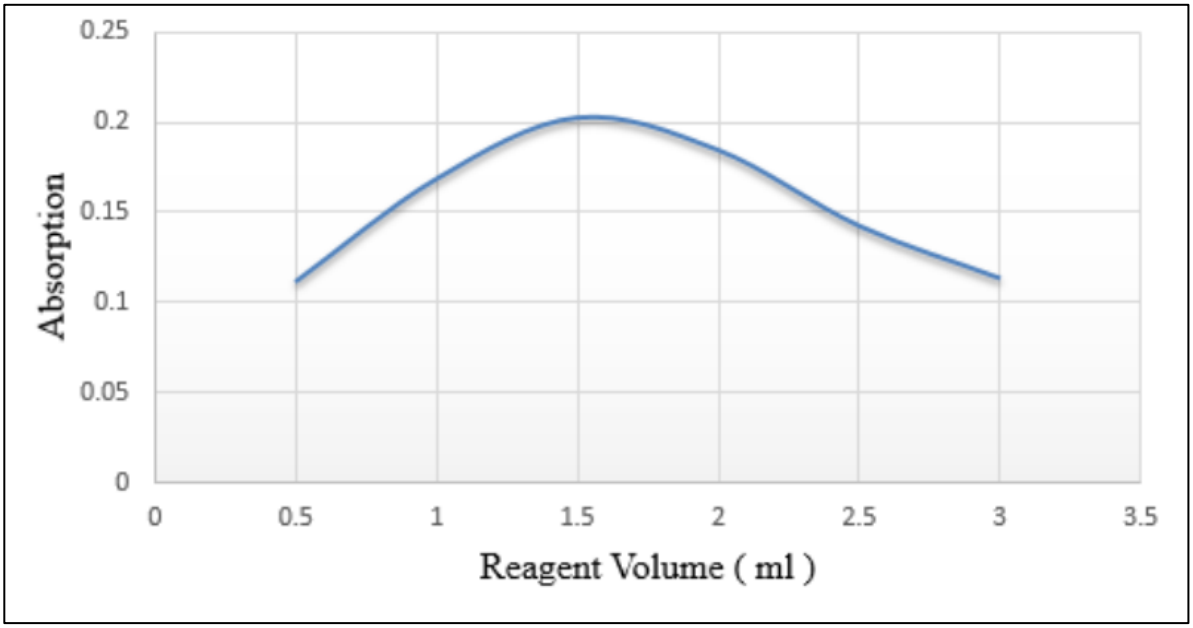


**Figure (5):** Effect of the volume of NQS 1.2% reagent on the absorption value of the resulting complex (gliclazide 11 µg/ml)

### 3.2.4. Effect of temperature:

Table (4) shows the absorption values of the derived complex after exposing the sample solution to different temperatures (12-12-24) degrees Celsius in a water bath with a heating time of (14) 92º. The table shows that the highest absorption value was achieved when the water bath was heated to (12) degrees Celsius.

**Table (4):** Effect of temperature on the absorption value of the complex resulting from derivatization (gliclazide 11 µg/ml)

| Water bath temperature (°C) | Heating time (minutes) | Absorption |
|---|---|---|
| 25 | 15 | 0.003 |
| | 60 | 0.001 |
| 60 | 15 | 0.050 |
| | 60 | 0.145 |
| 70 | 15 | 0.093 |
| | 60 | 0.163 |
| 80 | 15 | 0.115 |
| | 60 | 0.189 |
| 90 | 15 | 0.193 |
| | 60 | 0.201 |

### 3.2.5. Effect of heating time:

Table (5) shows the absorption values of the derived complex after heating the sample solution to 92°C for 12 minutes (32-24-22-14- in a water bath over increasing periods of time). We noted that the maximum absorption value was at a heating time of 22 minutes (Absorption 0.205) after which increasing the heating time did not have a positive effect on the absorption value.

**Table (5):** Effect of heating time on the absorption value of the complex resulting from derivatization (gliclazide 11 µg/ml)

| Heating time (minutes) | Absorption |
|---|---|
| 10 | 0.187 |
| 15 | 0.19 |
| 20 | 0.205 |
| 25 | 0.205 |
| 30 | 0.200 |
| 60 | 0.201 |

## 3.3. UV absorption spectrophotometry of gliclazide

Determining the maximum absorption wavelength of gliclazide in the ultraviolet range: Figure (6) shows that the maximum absorption wavelength of the gliclazide solution (12 µg/ml) 422 nm - 221 nm after conducting a spectral scan of it in the range (22).

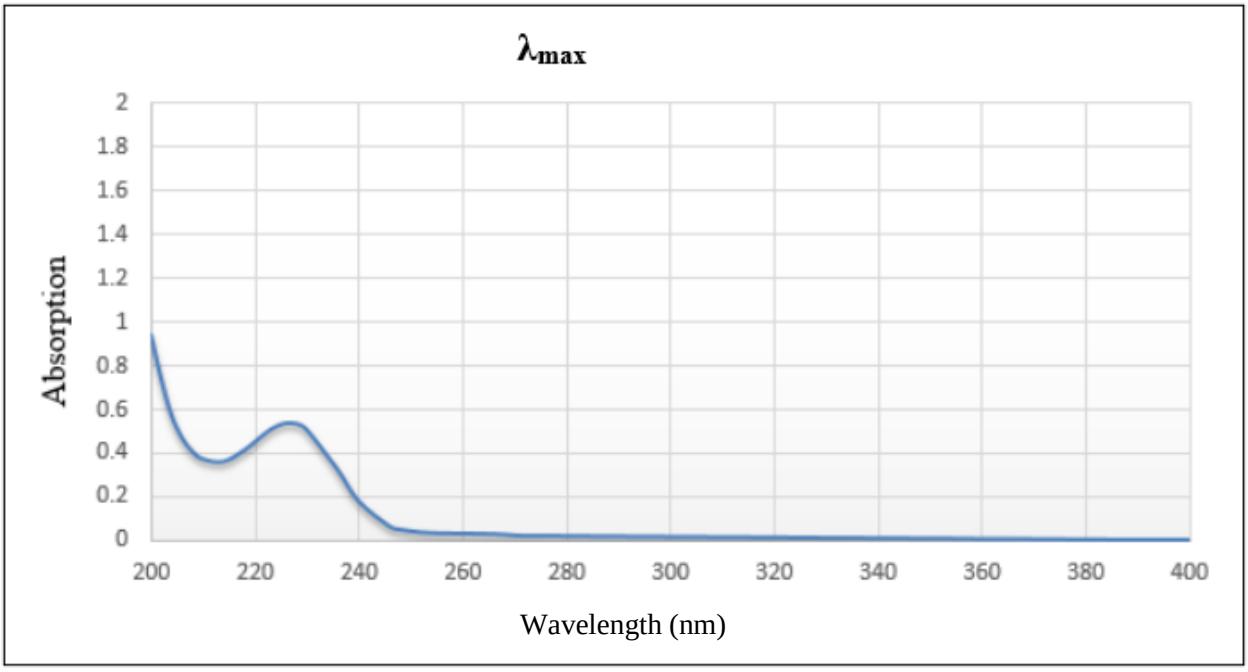

**Figure 6:** Maximum absorption wavelength of gliclazide solution in methanol (12 µg/ml)

## Gliclazide assay in pharmaceutical preparations:

Gliclazide assay in tablets based on the proposed chemical derivatization method using the Naphtoquinone-4-sulfonic acide sodium salt (NQS) reagent. Table 6 shows the results of the assay of gliclazide in tablets using the chemical derivatization method % (124.8 - 99.3)

**Table 6:** Results of the assay of gliclazide in pharmaceutical forms using the proposed derivatization method

| Pharmaceutical preparation | Theoretical amount of Gliclazide (mg/tablet) | Practical dosage of Gliclazide (mg/tablet) | Recovery % | Mean recovery ± SD | RSD |
|---|---|---|---|---|---|
| **Diamicron MR** | 60 | 59.9 | 99.33 | 100.06±1.65 | 1.65 |
| **Gliclazide Biomed** | 60 | 61.9 | 103.16 | 102.2±1.71 | 1.67 |
| **Bahri-Cron** | 60 | 61.8 | 103.13 | 103.1±1.19 | 1.15 |
| **Unicron** | 80 | 82.17 | 103.38 | 104±126 | 1.20 |
| **Meticron** | 80 | 79.59 | 99.48 | 99.35±1.12 | 1.13 |

The optimal conditions for complex formation between Naphtoquinone-4-sulfonic acide sodium salt (NQS) and glycazide were determined when testing the optimal solution, methanol showed the highest absorption value compared to acetonitrile and water (15). It was demonstrated that the use of water led to instability of the resulting derivatized complex, as observed by observing the change in the absorption value corresponding to the maximum absorption wavelength at each instant (16).

- Factors related to the reagent, such as concentration and volume, were studied. The concentration of 2.2 g/122 ml of NQS reagent in a volume of 1.4 ml showed the highest absorption value compared to other concentrations and volumes. Increasing the concentration and volume did not have a positive effect (17). Conversely, a decrease in the absorption value occurred. The reaction did not take place at laboratory temperature, and heating was necessary (18). A study of the effect of heating on the reaction revealed that the absorption was maximum when the sample was heated in a water bath at 92°C for 22 minutes. Increasing the heating time beyond 22 minutes, i.e., ºC, did not show a significant difference in the absorption values (19,20).

## 3.4. Results of Molecular Docking Studies

| Compound | Binding Affinity (kcal/mol) | Ligand Efficiency |
|---|---|---|
| Gliclazide | -7.7 | -0.35 |

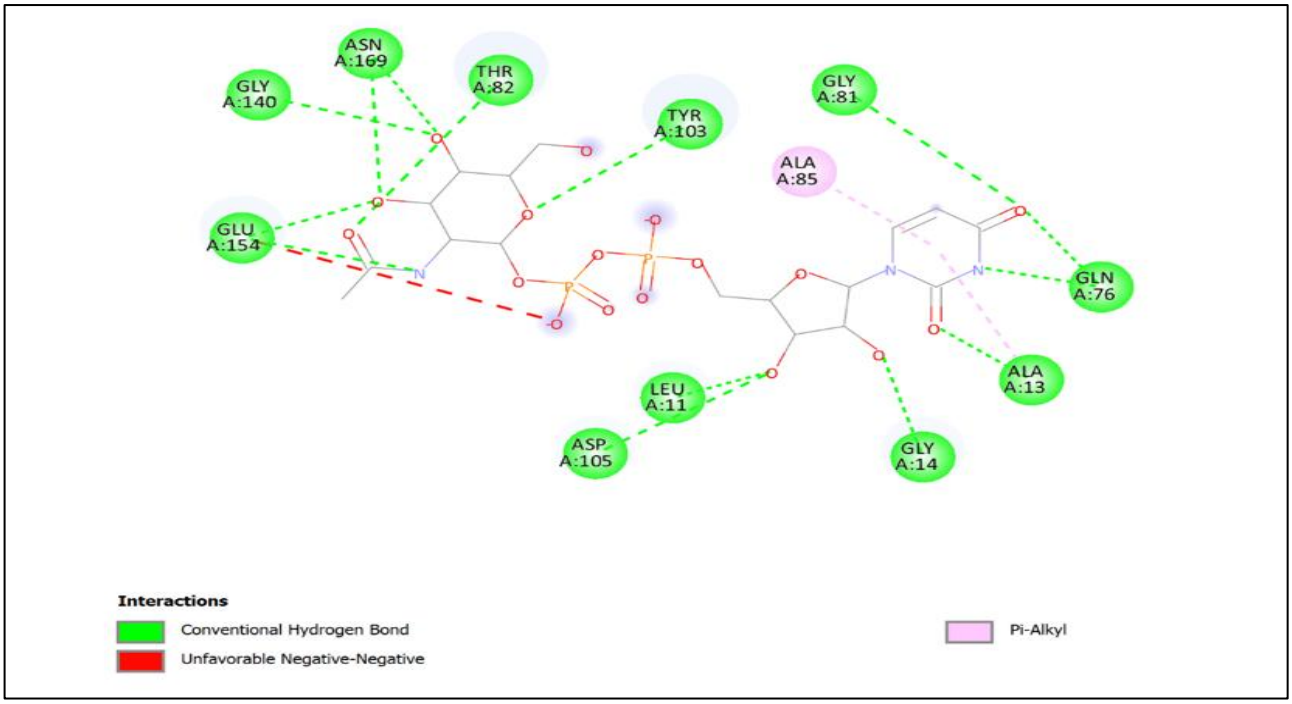


**Figure 7: The 2d active site cavity pose for Gliclazide.**

A prerequisite for effective and reliable molecular docking is the validation of the docking protocol. To avoid ambiguous results, the docking process was validated by docking the ligand, , into the defined binding site. According to the PDBsum database (27), the ligand formed interactions with the GlmU enzyme at the following amino acid residues: ASN169, GLU154, GLY140, THR82, GLY81, GLN76, ALA13, GLY14, LEU11, and ASP105.

For the docking study, the Gliclazide ligand was prepared using the PyRx program. After breaking any existing bonds and satisfying valences, the compound was docked and evaluated using AutoDock 4.2 based on binding energy. The binding affinity of Gliclazide, the native co-ligand of the GlmU enzyme, was recorded as –7.7 kcal/mol using AutoDock Vina, as shown in Table 7.

## CONCLUSION

1. An analytical method was developed for the assay of gliclazide in its pure form and in pharmaceutical preparations using the NQS reagent using a nucleophilic exchange mechanism in a methanolic medium with heat as a reaction medium. A colored complex was formed, the spectrum of which was scanned in the visible range.

2. The optimal conditions required for complex formation were studied, including the optimal solution, reaction temperature and heating time, and the concentration and a

mount of reagent to be added.

3. The use of a buffer had no positive effect, and the method was performed without extraction, confirming the ease and speed of the developed method.

4. The colored complex was stable for two hours, enabling the method to be used to assay a large group of samples.

5. The molar relationship of the complex was determined using the continuous variation method and the molar ratio method, where the binding ratio was 1:1. A reaction mechanism for the studied compound with NQS at the aforementioned binding ratio was proposed.

6. Using the Gliclazide molecule, the docking output revealed a potential potainial inhibitor with a binding affinity of -7.7 kcal/mol. These findings demonstrate the compound's capacity to block the UNC enzyme, which may help reduce and manage udder inflammation and maximize milk production.

**Funding**

None

**ACKNOWLEDGEMENT**

None

**CONFLICTS OF INTEREST**

The author declares no conflict of interest.

## REFERENCES

1. Skoog, A. D., et al. *Introduction to Spectrochemical Methods*. In: *Fundamentals of Analytical Chemistry*, 8th ed., pp. 710–715.
2. Harvey, D. *Spectroscopic Methods of Analysis*. In: *Modern Analytical Chemistry*, 2000, pp. 368–382.
3. Owen, T. (1996). *Fundamentals of UV-Visible Spectroscopy*, pp. 10–69.
4. Rouessac, F., & Rouessac, A. (2007). *Spectroscopic Methods*. In: *Chemical Analysis*, 2nd ed., pp. 167–176.
5. Adegoke, A. O. (2012). Chemical derivatization methodologies for UV-visible spectrophotometric determination of pharmaceuticals. *International Journal of Pharmaceutical Sciences Review and Research*, 14(2), 6–24.
6. Krupanandam, G. L. D., et al. (2004). Instrumental Methods of Analysis: Ultraviolet Spectroscopy. *Analytical Chemistry*, pp. 118–130. https://doi.org/10.2174/1573412917999200909144354
7. Hewlett-Packard Company. (1998). *Fundamentals of UV-Visible Spectroscopy: A Workbook*, pp. 56–71.
8. Nemcova, I., & Cermakova, L. (1996). *Principles of Analytical Spectrophotometry: Spectrophotometric Reactions*, p. 21.
9. Ravisankar, P., et al. (2017). Comprehensive review of important analytical reagents used in spectrophotometry. *Indo American Journal of Pharmaceutical Research*, 7(5), 2231–6876.
10. Darweesh, A. S., et al. (2013). Spectrophotometric determinations of sulfacetamide following simple diazotization and coupling with chromotropic acid. *Ibn Al-Haitham Journal for Pure and Applied Sciences*, 26(3), 281–295.
11. Pasha, C., & Narayana, B. (2010). A simple spectrophotometric method for the determination of mosapride by diazotization method. *Yanbu Journal of Engineering and Science*, 1(1431), 1658–5321.

12. Adegoke, A. O., & Quadri, O. M. (2016). Novel spectrophotometric determinations of some cephalosporins following azo dye formation with p-dimethylaminobenzaldehyde. *Arabian Journal of Chemistry*, 9, 1272–1282. https://doi.org/10.1016/j.arabjc.2012.02.005

13. Wafi, A., Supriyanto, G., & Tjahjandarie, T. (2015). A novel spectrophotometric method for determination of chloramphenicol based on diazotization reaction at room temperature. *Journal of Chemical and Pharmaceutical Research*, 7(3), 272–277.

14. Klokova, V. E., & Dmitrienko, G. S. (2008). Spectrophotometric determination of sulfanilamides by a condensation reaction with p-dimethylaminocinnamaldehyde. *Moscow University Chemistry Bulletin*, 63(5), 339–343. https://doi.org/10.3103/S0027131408050106

15. Adegoke, A. O., et al. (2014). Spectrophotometric determination of olanzapine after condensation with p-dimethylaminobenzaldehyde. *Journal of Taibah University for Science*, pp. 248–257. https://doi.org/10.1016/j.jtusci.2014.03.007

16. Sireesha, M., et al. (2011). Spectrophotometric determination of Nateglinide using 2,4-dinitrophenylhydrazine and potassium ferricyanide in pharmaceutical dosage form. *Der Pharma Chemica*, 3(1), 497–506.

17. Mandil, H., Sakur, A. A., & Al-Labban, A. (2015). Determination of esomeprazole and lansoprazole in pharmaceutical formulation by spectrophotometric analysis. M.Sc. Thesis in Analytical Chemistry, Aleppo University.

18. El-Didamony, A. M. (2008). Spectrophotometric determination of benzydamine HCl, levamisole HCl, and mebeverine HCl through ion-pair complex formation with methyl orange. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 69(3), 770–775. https://doi.org/10.1016/j.saa.2007.04.032

19. El-Didamony, M. A., Hafeez, M. S., & Saad, A. A. (2015). Application of bromocresol green and bromothymol blue for the extractive spectrophotometric determination of anti-hypertensive drugs. *Journal of Applied Pharmaceutical Science*, 5(7), 122–129. https://doi.org/10.7324/JAPS.2015.50719

20. Deperasinska, I., & Prochorow, J. (1977). Solvent effect on the charge-transfer absorption of molecular electron donor-acceptor complexes. *Institute of Physics, Polish Academy of Sciences*, 11(1–2), 51–61. https://doi.org/10.1016/0378-4487(77)80021-6

21. Kalas, V., Hibbing, M. E., Maddirala, A. R., Chugani, R., Pinkner, J. S., Mydock-McGrane, L. K., Conover, M. S., Janetka, J. W., & Hultgren, S. J. (2018). Structure-based discovery of glycomimetic FmlH ligands as inhibitors of bacterial adhesion during urinary tract infection. *Proceedings of the National Academy of Sciences*, 115(12), E2819–E2828. https://doi.org/10.1073/pnas.1720140115

22. Schrödinger, LLC. (2021). *The PyMOL Molecular Graphics System*, Version 2.5.

23. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, 30(16), 2785–2791. https://doi.org/10.1002/jcc.21256

24. O'Boyle, N., Banck, M., James, C., Morley, C., Vandermeersch, T., & Hutchison, G. (2011). Open Babel: An open chemical toolbox. *Journal of Cheminformatics*, 3(1), 33. https://doi.org/10.1186/1758-2946-3-33

25. Dallakyan, S., & Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. *Methods in Molecular Biology*, 1263, 243–250. https://doi.org/10.1007/978-1-4939-2269-7_19
26. Laskowski, R. A., Jabłonska, J., Pravda, L., Varekova, R. S., & Thornton, J. M. (2018). PDBsum: Structural summaries of PDB entries. *Protein Science*, 27(1), 129–134. https://doi.org/10.1002/pro.3289
27. Brown, K., Pompeo, F., Dixon, S., Mengin-Lecreulx, D., Cambillau, C., & Bourne, Y. (1999). Crystal structure of the bifunctional N-acetylglucosamine 1-phosphate uridyltransferase from *Escherichia coli*: A paradigm for the related pyrophosphorylase superfamily. *The EMBO Journal*.
28. Akash, M. S. H., Rehman, K., Akash, M. S. H., & Rehman, K. (2020). Ultraviolet-visible (UV-VIS) spectroscopy. Essentials of pharmaceutical analysis, 29-56.
29. Sarkar, A., Tiwari, A., Bhasin, P. S., & Mitra, M. (2011). Pharmacological and pharmaceutical profile of gliclazide: a review. Journal of Applied Pharmaceutical Science, (Issue), 11-19.