**Spectrophotometric determination of metformin in pharmaceutical preparation (tablets) and environmental water samples: Application to content uniformity testing**

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**Abstract**

A simple, accurate, and rapid visible spectrophotometric method has been developed for the determination of metformin in pure form, pharmaceutical preparations and environmental water samples . The method is based on the reaction of metformin with copper in alkaline solution in the presence of citrate ion to form a violet colored chromogen with an absorption maximum at 570 nm. Beer’s Law was obeyed in the range of 10-100 µg/ml with molar absorbitivity and Sandell,s sensitivity of 1.656×103 L.mol.-1.cm-1 and 0.1 µg/cm2 respectively. The relative standard deviation of the method was less than 2% and accuracy (average recovery ) was 100±1.4% .The optimum conditions for color development are described and the proposed method has been successfully applied for the determination of metformin in pharmaceutical preparations and water samples. The common excipients and additives did not interfere in the proposed method.

**الخلاصة**

تم تطوير طيفية تمتاز بالبساطة و السرعة والدقة العالية لتقدير المتفورمين في مستحضراته الصيدلانية (الحبوب) وفي نماذج بيئية (مياه) تعتمد الطريقة على تفاعل المتفورمين مع النحاس الثنائي في الوسط القاعدي وبوجود ايون السترات لتكوين معقد بنفسجي اللون له أقصى امتصاص عند 570 نانومتر وقد لوحظ أن قانون بير يسري على الكميات ألتي تتراوح بين 10 ـ100 مايكرو غرام /مل وان معامل الامتصاص المولاري ودلالة ساندل كانا 6.15x104 لتر\ مول.سم 0.99 نانوغرام \سم2 على التوالي. وان الانحراف القياسي النسبي للطريقة اقل من2% و الاسترجاعية 100±1.4 وقد تم دراسة الظروف المثلى للتفاعل وطبقت الطريقة بنجاح لتقدير المتفورمين في مستحضرات الحبوب وفي النماذج البيئية (مياه) وقد لوحظ عدم تداخل المواد الموجود مع مستحضرات الحبوب.

**Introduction**

Metformin hydrochloride (glucophage) (1) , chemically is 1,1-Dimethyl biguanide hydrochloride with a molecular formula of C4H12Cl N5 (Fig 1).



**Fig (1) : Chemical structure of metformin- HCl**

It is an oral antidiabetic drug that has been used in the treatment of non- insulin dependent diabetes which improves control of glycemia primary by inhibiting hepatic gluconeogenesis and glucogenolysis (2) and seems to ameliorate hyperglycemia by improving preipheral sensitivity to insulin , reducing gastrointestinal glucose absorption and hepatic glucose production. Recently, metformin has also become available for the treatment of polycystic ovary syndrom and has been found to improve vascular function , prevent pancreatic cancer and revers fatty liver diseases (3) .Literature survey reveals that many HPLC methods for the determination of metformin are reported . But most of the methods either using ion-pair reagent or cation exchange column(4–15). Another different methods for the determination of metformin have been described , such as conductometric titration(16) , flow-injection chemiluminescence(17-19), capillary electrophoresis(20), ion-selective electrode (21) and adsorptive catalytic squar-wave voltammetry (22) .Very few spectrophotometric methods for the determination of metformin hydrochloride in pharmaceutical formulation are available in the literature . The official method includes uv spectrophotometric method for estimation of the drug in tablets (23) . The colorimetric methods include charge transfer complex with iodine in acetonitrile medium (24) , reaction of metformin with Cu+2 in basic cyclohexyl amine medium (25) and the reaction with ninhydrin to form a violet colored complex(26). And spectrophotometric method using multi variate technique (27). However all of these methods suffered from several disadvantages including use of complex extraction procedures which were tedious and time consuming. The proposed method is simple and applicable as well as for routine analysis of metformin hydrochloride in tablets and environmental water samples.

**Experimental**

**Apparatus**

A Genway 6405 Uv / visible spectrophotometer with 1.0 cm quartz cells was used .

# Reagents

All chemical used were of analytical or pharmaceutical grade and the metformin hydrochloride standard material was provided from state company of drug industries and medical appliance(NDI) Ninavah - Iraq .

**Metformin hydrochloride standard solution (500 ppm)(3x10-3 M).**

This solution was prepared by dissolving 0.05 gm of metformin hydrochloride in 100ml distilled water in a volumetric flask .

**Sodium hydroxide solution (1N).**

**Copper sulfate penta hydrate solution (3x10-2 M).**

This solution was prepared by dissolving0.75 gm of reagent in100ml of distilled water in a volumetric flask .

**Citric acid solution (0.2M).**

This solution was prepared by dissolving 3.84 gm of reagent in100ml of distilled water in a volumetric flask.

**General procedure**

Aliquots of standard solution of metformin hydrochloride (0.25-2.5mg) were transferred into a series of 25ml calibrated flasks, added 5ml of copper sulfate solution,5ml of citric acid solution and 5ml of 1N sodium hydroxide , dilute the solution to the mark with distilled water . The absorbance of the violet-colored products was measured at 570 nm against a reagent blank.

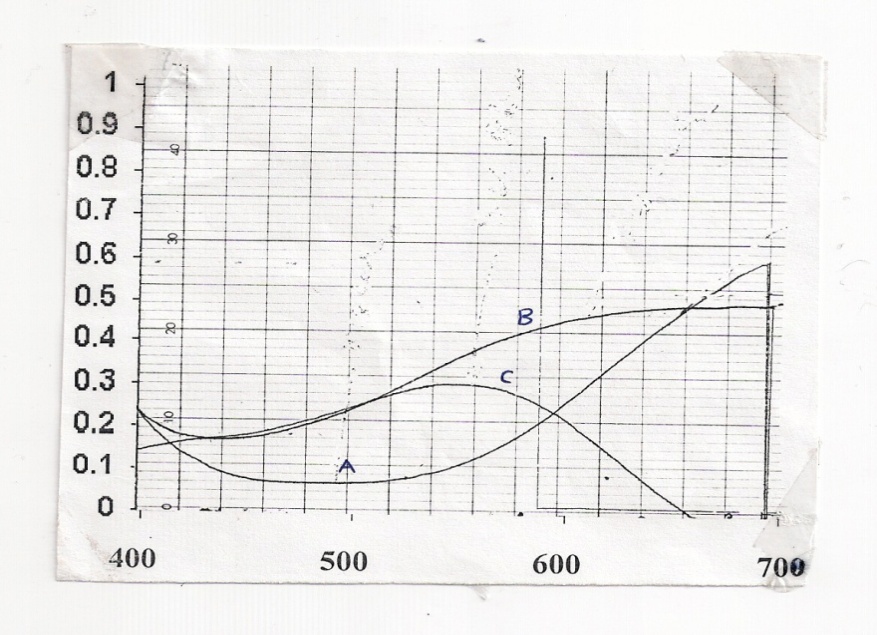
**procedure for pharmaceutical preparations (tablets)**

Weight and powder 10 tablets . Dissolve a quantity of the powdered tablets equivalent to 0.05 gm of metformin hydrochloride in about 70ml distilled water and mixed for 20 mint and then filtered. The filtrate was mad up to 100ml with distilled water . Treat 3ml of this solution as mentioned under general procedure.

**Procedure for water samples**

Distilled and tap water samples (100ml)were fortified with 0.05 g of metformin hydrochloride .The fortified water samples were analyzed as desired under general procedure .

**Results and Discussion**

 The reaction between metformin and CuSO4 in alkaline medium.The reaction was carried out in the presence of citrate ion to prevent copper precipitation yields a violet color complex , which absorb at 570nm fig(2).

**Fig(2):Absorption spectra of metformin hydrochloride (25µg/ml) and its complex with copper. A-copper sulfate against water .B-metfomin-copper complex against water .C- metfomin-copper complex against copper sulfate solution.**

Wavelength ()

Various experimental parameters affecting the development and stability of the reaction product was optimized by changing each variable in turn while keeping all other variables constant

**Effect of bases**

The preliminary quantitative examination of color reaction between metformin and copper indicated that a characteristic violet color of the complex was formed only in alkaline solution .So that different volume of1M of different bases (NaOH, KOH, Na2CO3,NH 4OH) have been tested for this purpose. NaOH and KOH gives high sensitivity than the others. Then 5ml of 1M NaOH solution was selected subsequent work.

**Effect of citric acid solution**

The amount of citric acid 0.2 M solution for maximal color intensity was examined . The maximum constant intensity was reached at 3 ml and remained constant up to 9 ml.However,5ml of citric acid solution was selected for subsequent work. **Effect of copper sulfate solution**

The amount of copper sulfate 0.03 M solution for maximal color intensity was examined . The maximum constant intensity was reached at 3 ml and remained constant up to 9 ml.However,5ml of reagent solution was selected for subsequent work. **Effect of reaction time**

The maximum time for complete color development of the complex was found to be 5 min at room temperature. The color was then stable for at least 24 hours.

**Order of the addition of reagents**

To test the effect of order of addition of the reagents on absorbance ,different orders were tested .The selected order was sample solution ,copper sulfate, citric acid ,followed by NaOH solution, because of its high absorbance value.

**Beer,s law**

Under the recommended conditions described above a liner calibration graph for metformin hydrochloride within concentration range of 10-100µg/ml ,with correlation coefficient of 0.998, intercept of 0.003 and slope of 0.010. Fig(3) was obtained.

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**Fig(3):- Calibration graph of metformin hydrochloride.**

**Accuracy and precision of the proposed method.**

To evaluate the accuracy and precision of the methods a pure drug solution was analyzed at three different concentrations, each determination being repeated six times. The relative error (%) and relative standard deviation (RSD) values are summarized in table (I). From table (I), it is clear that relative error of ≤ 1.4 % is as accurate Moreover, the method was found to be precise with RSD values <1.9 %.

**Table I ; Accuracy and precision of the proposed method.**

|  |  |  |
| --- | --- | --- |
| Metformin hydrochloride taken (mg) | Er(%)a | RSD% |
| 0.025 | 1.4 | 1.6 |
| 0.05 | 1.3 | 1.8 |
| 0.1 | 1.1 | 1.8 |

a: Mean of six determinations

**Interferences**

The interfering effects of foreign species that often accompany with metformin hydrochloride in pharmaceutical preparations were studied by adding different amounts of foreign species to 30g/ml of metformin hydrochloride in solution and the general procedure for the determination of metformin hydrochloride was followed. The species was considered not to interfere if it caused a change of less than 2% in the absorbance obtained for metformin hydrochloride alone (28) . It was observed that the starch, Lactose, magnesium stearate , methyl hydroxy benzoate and propyl hydroxy

benzoate, don`t interfere with the determination method at levels found in dosage form. So that the selectivity of method was very good

**eactionStoicheiometry of the r**

The stoicheiometry of the reaction between metformin and copper in presence of citrate was investigated using job's method(continuous variation), of equimolar solution(3x10-3M), the result obtained show that 1:1:1 metformin-copper-citrate at 570 nm fig(4) and the suggested reaction and structure of the product might be written as .



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**Fig(4) :Continuous variation plot for reaction of metformin.HCL with Cu(II).**

**Analytical applications**

The proposed method was satisfactory applied to the determination of metformin hydrochloride in its pharmaceutical formulations and water samples . the results of the assay of the pharmaceutical formulations revels that there is close agreement between the results obtained by the proposed method and the lable claim. The results were also compared statistically by student t-test and by the variance ratio F-test with those obtained by uv- spectrophotometric official method (23) at 95% confidence level .The calculated t- and F- values did not exceed the theoretical values indicating that there was no significant differences between the precision of the proposed and literature method as cited in table( 2) , And the results of water samples table (3) show that the recovery values obtained were close to 100%.

**Table(2): Determination of metformin hydrochloride in pharmaceutical formulations**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Pharmaceutical formulations | Lable amount mg | Found by proposed method mg\* | official BP method(23) | t value | F value |
| Tablets | 500mg/tab | 498.92 | 499.95 | 1.34 | 1.62 |
| Tablets | 850mg/tab | 850.58 | 850.71 | 1.98 | 1.56 |

\*mean value of ten determinations

T values (n=10, at 95% confidence level tabulated value 2.262).

F values (n1-1 and n2-1 =9, at 95% confidence tabulated value 3.18).

**Table[3] : Determination of metformin hydrochloride in environmental water sample*s***

|  |  |  |  |
| --- | --- | --- | --- |
| **% Recovery(n=10)** | **Metformin hydrochloride (mg/ml)\*** | | **Water samples** |
| **Found** | **taken** |
| 99.4 | 9.94 | 10 | Tap water |
| 100.00 | 30.0 | 30 |
| 99.8 | 49.9 | 50 |
| 99.6 | 9.96 | 10 | River water |
| 99.66 | 29.9 | 30 |
| 100.00 | 50.0 | 50 |

\*Mean of ten determinations.

**Application of the method to content uniformity**

The proposed method proved to be suitable for the content uniformity test, where a great number of assays on individual tablets are required. Data presented in Table[4] indicate that the proposed method can accurately and precisely quantitate metformin hydrochloride in its commercially available tablets. The mean percentage (with RSD) of the labeled claim found in ten tablets was (0.54%) which fall within the content uniformity limits specified by the USP 30 29. The proposed method may be regarded comparable to someexisting methods(25,26,30-31) ,as shown in table[5].

**Table[4]:Content uniformity testing of metformin hydrochloride tablets using the proposed method**

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| Parameter | % of the label claim |
| Tablet NO. 1  Tablet NO. 2  Tablet NO. 3  Tablet NO. 4  Tablet NO. 5  Tablet NO. 6  Tablet NO. 7  Tablet NO. 8  Tablet NO. 9  Tablet NO. 10  Mean ( x )  % RSD  Max. allowed unit (29) | 100. 25  100. 03  99. 56  100. 73  99.38  99. 35  99.72  100. 52  100.66  99.71  100. 48  0.54  ±15% |

**Table[5]:Comparison the proposed method with some spectrometric methods**

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| --- | --- | --- | --- | --- | --- | --- |
| RSD | %Recovery | Sandell,s sensitivity µg/cm2 | ɛ =L/ mol.cm | Beer s law range (µg/ml) | maxλ  (nm) | MethodsRef |
| ————-1.73  3.12  Less than 2 | 97-100  ————  100±0.5  ————  100±1.4 | 0.17  ————————  0.046  0.1 | 5.7x103  ————  ————9.9x103  1.165x103 | 8-18  500-2000  2-10  4-24  10-100 | 570  540  237  240  570 | 26  25  30  31  Proposed method |

**Conclusion**

The proposed method was simple, accurate, precise, and low economical cost. Furthermore, the proposed method doesn’t require elaboration of procedures, which are usually associated with chromatographic methods. The proposed method could be applied successfully for determination of metformin hydrochloride in pure form , in tablet dosage forms as well as in environmental water samples ..

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