Facial visible spectrophotometric determination of metformin hydrochloride in glucosam tablets and industrial waste water: Application to content uniformity testing

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ABSTRACT

Objectives: To determine simple, accurate and highly sensitive spectrophotometric method for determination metformin hydrochloride in pharmaceutical preparations and in industrial waste water sample.

Materials and Method: The method is based on the oxidation of metformin by a known excess of sodium hypochlorite in alkaline medium.

Results: Formation of a yellow-colored chromophore having maximum absorbance at 385nm. Molar absorptivity was found to be 2×10^4 $L.mol^{-1}.cm^{-1}$. Beer's law is obeyed in the concentration range of 0.5-4 µg $.ml^{-1}$, relative standard deviation (RSD) is better than ±1.8 (n =10) .The limits of detection and quantitation are 0.083 and 0.25 µg $.mL^{-1}$, respectively. The method is applied successfully to determination of metformin pharmaceutical formulation (tablets).The common excipients do not interfere with the proposed method. A statistical comparison of these results with those of official method using t and F values at 95% confidence level shows good agreements and indicated no significant difference in the precision, and the present method has good validity.

Conclusion: The proposed method can be used as a routine quality control and content uniformity tests for determination of metformin in pure form, tablet formulations and industrial waste water sample.

Keywords: Metformin hydrochloride, spectrophotometry, content uniformity.

الخلاصة

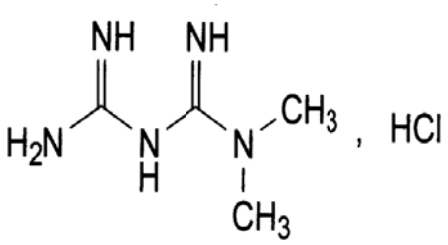
الهدف: تم تطوير طريقة بسيطة ذات دقة وحساسية عالية لتقدير الميتفورمين هيدروكلورايد في بعض مستحضراته الدوائية وفي نموذج من المياة الصناعية المطروحة .

المواد وطرق العمل: تعتمد الطريقة على أكسدة الميتفورمين بواسطة هايبوكلورات الصوديوم في وسط قاعدي.

النتائج: تكوين ناتج كروموجيني أصفر اللون له أقصى امتصاص عند 385 نانوميتر وبامتصاصية مولارية2×10^4 لتر.مول$^{-1}$.سم$^{-1}$. وقد لوحظ أن قانون بير يسري على الكميات التي تتراوح بين 0.5- 4.0 مايكروغرام /مل . إن الانحراف القياسي النسبي للطريقة احسن من± 1.8% وان حدي الكشف والكمي للطريقة هما 0.083 و 0.25 مايكرو غرام /مل على التوالي. استخدمت الطريقة بنجاح لتقدير الميتفورمين في الحبوب وان المواد الموجودة مع المستحضر لا تتداخل في هذه الطريقة وقد أختبر نجاح الطريقة بمقارنتها مع الطريقة القياسية الدستورية المعتمدة باستخدام اختباري (t) و(F) عند حدود ثقة 95%

الاستنتاج: يستدل على صلاحية التطبيق التحليلي للطريقة في التحليل الروتيني وفي السيطرة النوعية لتقدير الميتفورمين بحالته النقية وفي مستحضر الحبوب وكذلك في نموذج من المياة الصناعية المطروحة

Metformin hydrochloride (glucophage)[1], chemicals 1,1-Dimethyl biguanide hydrochloride with a molecular formula of $C_4H_{12}Cl N_5$ (Fig 1). It is an oral antidiabetic drug that has been used in the treatment of non- insulin dependent diabetes which improves control of glycemia primarily by inhibiting hepatic gluconeogenesis and glycogenolysis[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].



M. Wt = 165.6

Fig 1. Chemical structure of metformin- HCl.

Literature survey reveals that many HPLC methods for the detremination of metformin are reported. But most of the methods used either 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 chemilumine-scence[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 described. The official method includes UV spectrophotometric method for estimation of the drug in the tablets[23]. The colorimetric methods include charge transfer complex with iodine in acetonitrile medium[24], reaction of metformin with Cu^{+2} in basic cyclohexylamine 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, ultra filtration and column-switching technique, have been suggested to improve specificity and selectivity. The proposed method can be applicable to routine analysis and content uniformity test of metformin hydrochloride in tablets and complies well with the validation requirements in the pharmaceutical industry [28].

Material and methods

Apparatus

A spectro scan 50 UV visible spectrophotometer with 1.0 cm quartz cells was used .

Reagents

All chemicals used were of analytical grade and the metformin hydrochloride standard material was provided from

Ninevah drug industry and medical appliance (NDI), Iraq.

Metformin hydrochloride stock solution (100 ppm) was prepared by dissolving 0.1 g of metformin hydrochloride in 1L distilled water in a volumetric flask.

Metformin hydrochloride standard solution (25 ppm) was prepared by diluting 25 mL of stock solution to 100 mL by distilled water in a volumetric flask.

Sodium hypochlorite solution (0.1%) was prepared by dilution 1.25 mL of 8% sodium hypochlorite to 100 mL by distilled water, this solution was standardized every 4-5 days and stored in a dark bottle[29].

Sodium hydroxide solution (10 N)

Recommended procedure

Aliquots of standard solution of metformin hydrochloride (12.5-100 µg) were transferred into a series of 25 ml calibrated flasks, 5 mL of 10 N sodium hydroxide and 2 mL of 0.1% sodium hypochlorite solution, and the solution was diluted to the mark with distilled water . The absorbance of the yellow-colored product was measured at 385 nm against a reagent blank.

Procedure for pharmaceutical preparations (tablets)

Weigh and powder 10 tablets . Dissolve a quantity of the powdered tablets containing 0.025 g of metformin hydrochloride in about 100 mL distilled water and mix for 20 min and then filtered. The filtrate was made up to 1L with distilled water. Treat 3 ml of this solution as mentioned under recommended procedure.

Procedure for industrial waste water

To demonstrate the practical applicability of the proposed method, industrial waste water sample from the state company for drug industries and medical appliances, Mosul, Iraq, were analyzed by spiked with the concentrations ranging from 0.5-3.0 $\mu g.mL^{-1}$ of metformin hydrochloride and aliquot of this solution was treated as described above for recommended procedure.

Results and discussion

Metformin hydrochloride is oxidized in alkaline medium by sodium hypochlorite solution forming a yellow-colored chromophore which absorbs maximally at 385 nm as shown in Figure 2. The colorless reagent blank has practically negligible absorbance at this wavelength and this wavelength was recommended for determination.

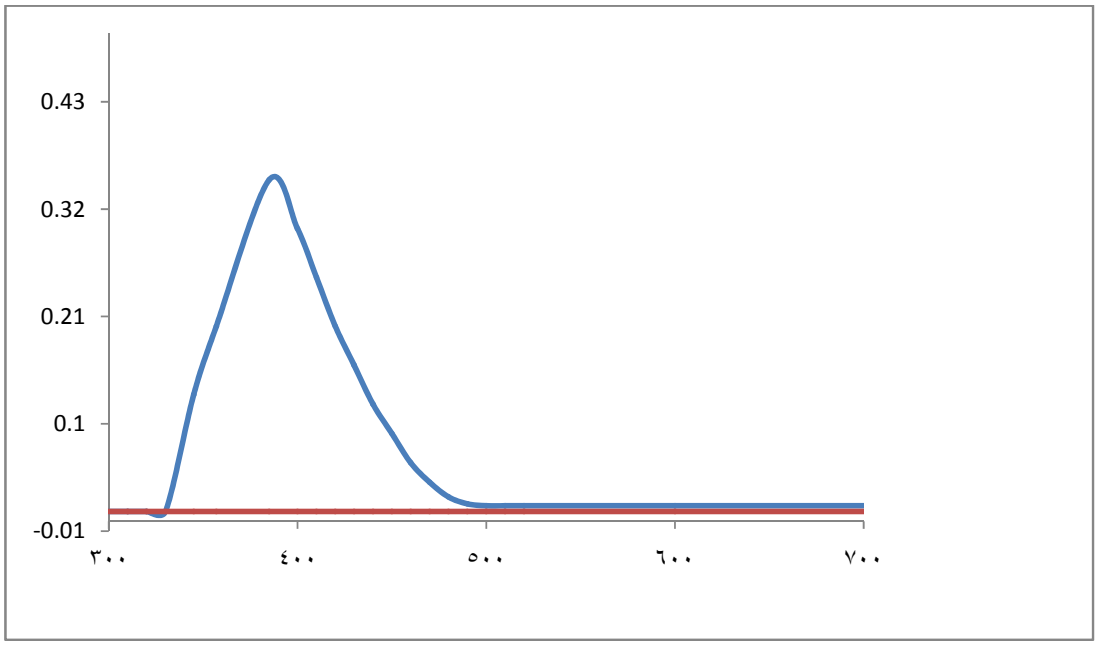


Figure 2. Absorption spectra of metformin hydrochloride product (B) against blank (A), metformin hydrochloride taken (75µg/ 25 mL)

The reaction variables were optimized by varying each variable, while keeping others constant for obtaining maximum absorbance. The oxidation reaction was found to be quantitative in sodium hydroxide medium .It was found that 5 ml of 10 N sodium hydroxide solution give high sensitivity and this amount has been used for subsequent experiments . The effect of the amount of sodium hypochlorite on the absorbance was investigated. A maximum and constant absorbance was found with 1-5 ml of 0.1% sodium hypochlorite solution and 2.0 ml has been used for subsequent experiments . The color reaction occurred at room temperature immediately and remained stable for at least six hours and a reaction time of 5 min was selected for reproducible results. Under the experimental conditions described, Beer's law is obeyed over the concentration range 0.5-4.0 µg/mL (Figure 3)

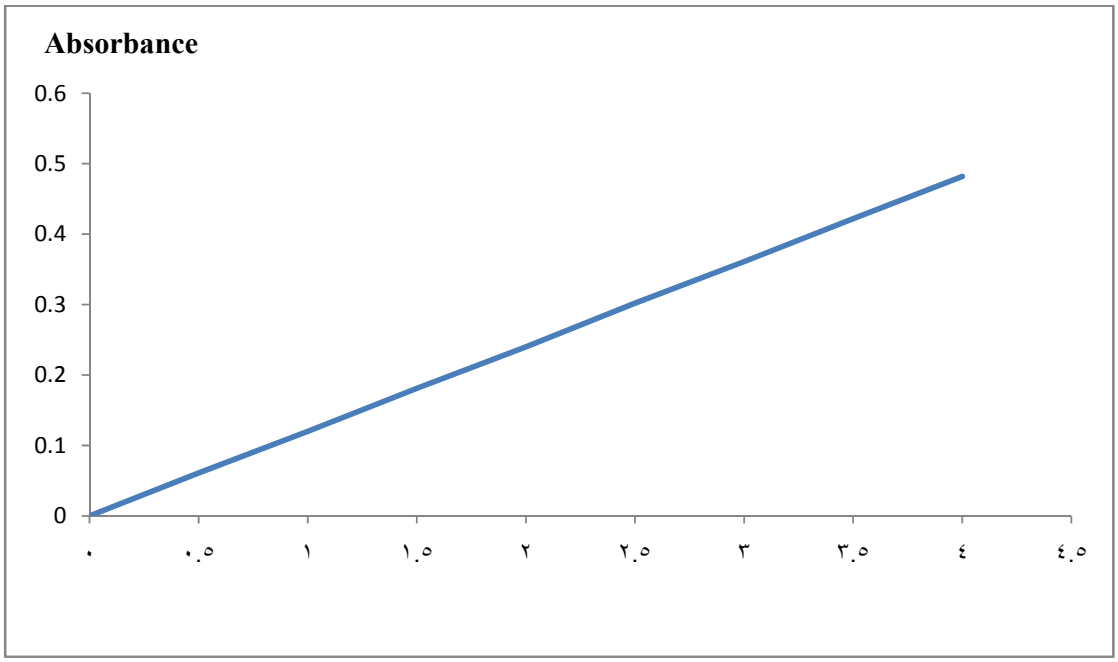


Figure 3. Calibration graph of metformin hydrochloride

A regression analysis of Beer 's law plot at 385 nm revealed a good correlation (r=0.9999, n=8) the graph of the absorbance versus the concentration of metformin hydrochloride showed a low intercept (6.097×10^{-6}) and slope (0.120) and is described by a regression equation Y = ax + b (where x is the concentration of metformin hydrochloride in µg/mL, the absorbance is Y, the slope is a and the intercept is b. The apparent molar absorptivity was 2×10^4 $L.mol^{-1}.cm^{-1}$ .The limit of detection and quantification were evaluated [30].

$$LOD = 3.3 \frac{So}{b}$$

$$LOQ = 10 \frac{So}{b}$$

Stoichiometry of reaction

The stoichiometry of reaction was investigated by the mole ratio method[31]. The results obtained indicated the existence of 1:6 metformin hydrochloride – sodium

Where b is the slope and So is the standard deviation of the regression line. The limit of detection was 0.083 µg $.ml^{-1}$ and limit of quantification(as the lowest standard concentration which could be determined with acceptable accuracy and precision) was 0.25 µg $.mL^{-1}$.

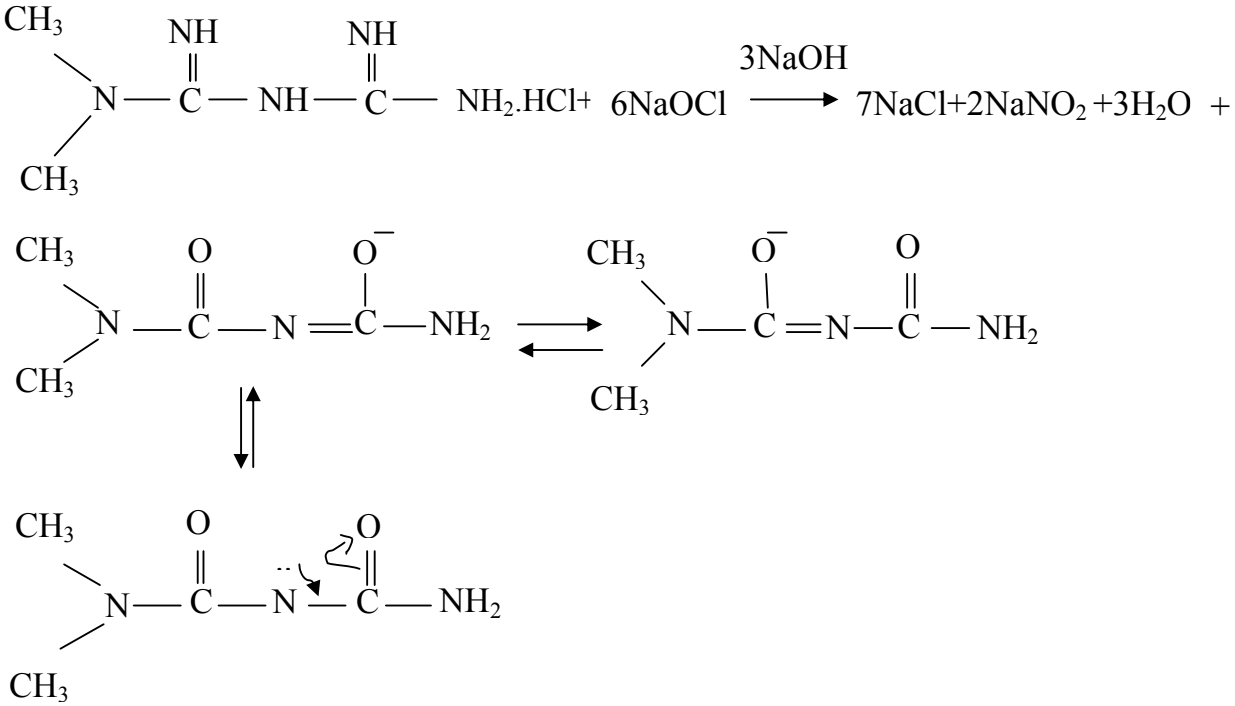
Accuracy and precision

The accuracy and precision of the method was established by analyzing the pure drug solution at three different levels. The average recovery which is a measure of accuracy was 100±0.73 revealing high accuracy of the method. The relative standard deviation (RSD), which is the indicator of precision is better than ±1.8% . The results are complied in Table 1.

hypochlorite. Thus the suggested reaction might be written as in other study[32].

Table 1. Optical characteristics and statistical data for regression equation of the proposed method

Parameters	Value
λ max (nm)	385
Beer´s law limits (µg .ml^{-1})	0.5 – 4.0
Molar absorpitivity (1.mol^{-1}.cm^{-1})	2.0×10^4
Limit of detection (µg .ml^{-1})	0.083
Limit of quantification (µg .ml^{-1})	0.25
Determination coefficient (r^2)	0.9999
Regression equation (Y= a × + b)	
Slope (a)	0.125
Intercept (b)	6.097×10^{-6}
Recovery %	100 ± 0.73
Relative standard deviation (%)	< 1.8



β-diketone yellow- chromogen

Effect of interferences

The interfering effect of foreign species often accompanied with

metformin hydrochloride in the pharmaceutical preparations were studied by adding different amounts of foreign species to 75 µg/25ml of metformin hydrochloride in solution and the recommended procedure for the determination of metformin hydrochloride was followed. The species are considered to interfere seriously if the cause aching of more than 2% in the absorbance obtained for metformin hydrochloride a lone[33]. Results of the recovery analysis are presented in Table 2. Excipients at the concentration show in Table [2] do not interfere with the assay .In addition recoveries in most cases were around 100% .

Table 2. Determination of metformin hydrochloride in the presence of excipients

Excipients	Amount taken, (µg/ml)	Average recovery, * %
Talc	500	99.98
	1000	100.06
Mannitol	500	100.09
	1000	99.92
Mg – stearate	500	100.05
	1000	100.03
Starch	500	100.06
	1000	100.03
Microcrystalline cellulose	500	99.92
	1000	99.90

* Average of five replicate determinations .

Application of the proposed method

The proposed method was successfully applied to the analysis of metformin hydrochloride in tablets and industrial waste water sample. The result of analysis for pharmaceutical formulations Table 3 were compared statistically by student t-test and by variance ratio F-test with those obtained by 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 difference between the precision of proposed and official methods. The results of waste water sample Table 4 show that the recovery values obtained were closed to 100%.

Table 3. Assay of metformin hydrochloride in pharmaceutical formulations

Pharmaceutical formulation supplied by NDI	Amount of metformin hydrochloride *		t value	F value
	Proposed method	Official method		
Glucosam tablets (500 mg/ tablet)	498.9	496.8	1.74	2.44
Glucosam tablets (850 mg/ tablet)	849.1	850.1	1.92	2.31

*Mean of ten determinations, t values (n=10) at 95% confidence level , tabulated value =2.262, F values (n1and n2 =10) at 95% confidence level , tabulated value =3.18

Table 4. Determination of metformin HCl in industrial waste water sample

Water sample	Metformin .HCL (µg /ml) * Taken	Found	% Recovery (n=10)
Industrial waste water	0.5		100
	0.5		101
	1.0		100.6
	1.01		
	3.0	3.02	

*Mean of ten determinations

Table 5. Content uniformity testing of metformin hydrochloride tablets using the proposed method

Parameter	% of the label claim
Tablet NO. 1	100.52
Tablet NO. 2	100.73
Tablet NO. 3	99.35
Tablet NO. 4	100.48
Tablet NO. 5	99.38
Tablet NO. 6	99.56
Tablet NO. 7	99.72
Tablet NO. 8	100.25
Tablet NO. 9	100.66
Tablet NO. 10	99.71
Mean ($\bar{x}$)	100.03
% RSD	0.54
Max. allowed unit [28]	±15%

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 5 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 [28].

Conclusion

In this work, a simple, rapid, precise and accurate spectrophotometric method was developed and validated for the determination of metformin hydrochloride in pharmaceutical preparations and industrial waste water samples. The method free from such experimental variables as heating or solvent extraction step. The method rely on the use of simple and cheap chemicals and techniques and can be used for rapid routine determination and quality control of metformin hydrochloride in pure form, bulk sample, pharmaceutical preparations and real industrial waste water sample.

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