# Continuous Flow Injection Analysis (CFIA) of Metformin Hydrochloride using Microphotmeter Equipped with 530 and 550nm LED.

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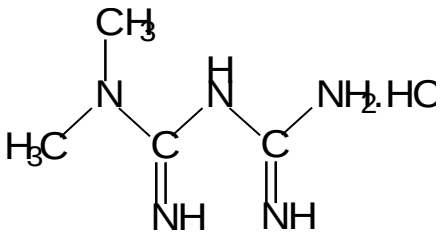
**Abstract**

Newly developed spectrophotometric method was used in the present research project for the determination of metformin hydrochloride drug (MTF.HCl), via the complexation of the drug with copper(II). The colored products was measured at 530 nm. The optimization of all chemical and physical parameters for MTF- $OH^-$ - Cu(II) system are described. A linear range of 94.04 % for 0.0-100 mM was obtained with a Limit of Detection (L.O.D) 662 ng. The newly developed system was applied for the analysis of pharmaceutical preparation. A comparison was made using paired t-test shows that, the newly developed method can be used as an alternative analytical method for the analysis of metformin hydrochloride (MTF). All this project work was based on on-line determination via Continuous Flow Injection Analysis (C.F.I.A).

Keywords: metformin.HCl; flow injection; spectrophotometry; pharmaceuticals.

## 1. Introduction

Among the various substituted biguanides, Metformin. HCl (Fig.(1)) (MTF.HCl; N,N-dimethyl biguanide hydrochloride)[1,2] is one of the interesting compounds that chelate metals. It is an antidiabetic agent which uses in the treatment of non-insulin- dependent diabetes mellitus (NIDDM)[3,4], it also acts as antimicrobial, analgesic, antimalerial[5] and antimetabolite for organisms that inhibit the metabolism of folic acid[6]. Various methods have been developed for the determination of MTF.HCl in pharmaceutical preparations and biological samples including Potentiometric titration[7], Spectrophotometry [8,9], Gas Chromatography[10,11] and Liquid Chromatography[12].




(10)

*Fig.(1) Structure of MTF.HCl.*

A method based on the flow injection (FI) is a well known technique that offers improvement in most batch methods, providing high sample throughput rate, simple sample preparation and instrumentation [13].

The aim of this work is to develop a very simple and sensitive continuous-flow method for the determination of metformin via the formation of colored complex with Cu(II) ion in alkaline medium (metformin-$OH^-$-Cu(II)) using a light emitting diode (LED) based spectrophotometric detection and the colored product was measured at 530 nm. The newly developed system was applied for the analysis of pharmaceutical preparation. All the results will be subjected for detailed data treatments and comparison with other available methods.

## 2. Experimental

### 2.1. Chemicals

A stock of Metformin.HCl solution (200 mM) was prepared by dissolving 16.563 g of MTF.HCl in distilled water. A copper(II) solution (1000 $\mu g.ml^{-1}$) was prepared by dissolving 7.6046 g ($Cu(NO_3)_2.3H_2O$) (BDH)) in distilled water and diluting to 2000 ml. A stock solution sodium hydroxide (100mM) was prepared by dissolving 4.0 g of NaOH (BDH) in distilled water and diluting to 1000 ml, which was standardized with standard hydrochloric acid.

### 2.2. Apparatus

The flow injection manifold is shown in Fig.(2). It consists of a peristaltic pump (An ISMATEC type ISM796, Switzerland) and the sample is injected via a V-450 (upchurch scientific Inc.) rotary six ports injection valve with a variable sample loop. Poly propylen or teflon tubing with inside diameter between 0.5-1 mm were used. Liquid junction point made of polymethylmethacrylate (organic

glass) (Y-junction) for the combination of the streams; there are two Y-junction, the first junction were the carrier stream, carrying the metformin drug meet the hydroxide ion and directly mixes with the Cu(II) ion at the second Y-junction. The flow cell is a hexagonal 2 mm path length with a photosilicon detector will complete amplification and control for intensity of the incident light; thus insure the possibility of variation of the power of the incident light with variable of concentration of reactant and two high intensity light emission diod (LED)[14] of a maximum wave length 530 and 550 nm were used. The output can be represented digitally or by plotting on X-t recorder or both at the same time.

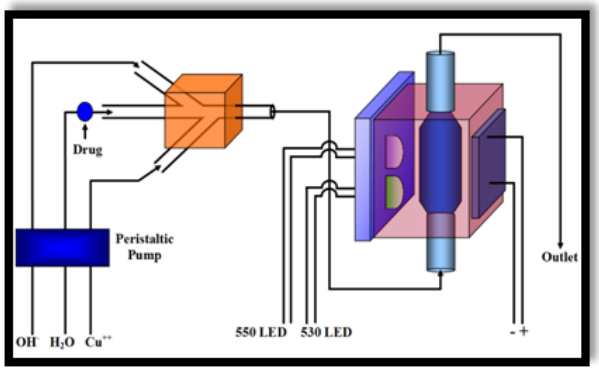



***Fig.(2) FI manifold for the determination of MTF.HCl.***

## 3. Results and Discussion

### 3.1. Order of addition

It was found that whether drug treated with alkaline solution followed by the solution of Cu(II) ion gave a clear pink color compared with other combination of the order for the addition of the chemicals as shown in Table (1), therefore a reaction system was designed on this basis.

***Table (1)***
***Order of addition of chemicals used in metformin formation Complex.***

| *Order of addition* | *Color of the reaction product* |
|---|---|
| A+B+C | Pink(faint) |
| B+A+C | |
| A+C+B | Violet turns into pink at an excess $OH^-$ ion |
| C+A+B | |
| B+C+A | Violet turns into purple at an excess drug with less intensity of the pink color |
| C+B+A | |

**** No color is formed when any two combinations is carried out. A=MTF.HCl; B=$OH^-$ ; C=Cu(II).***

### 3.2. Spectrophotometric scanning for the MTF-$OH^-$-Cu(II) system

A scanning were carried out from 200-800 nm for three chemicals, metformin, Cu(II) ion and pink colored complex for MTF-$OH^-$-Cu(II) system as shown in Fig.(3). These experiment were very necessary in order to design the detection unit with a light emitting diod (LED) at a certain and selected wave length suitable for the measurement of the colored species.

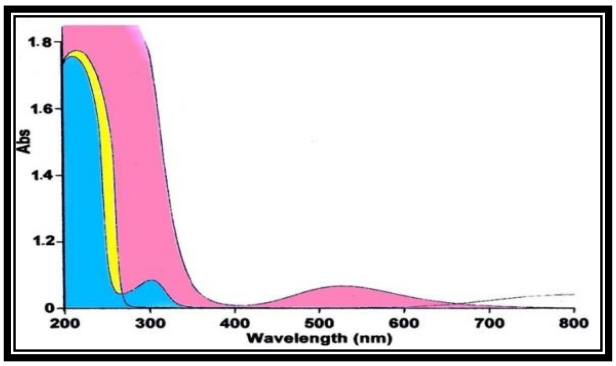



***Fig.(3) UV-Vis spectrophotometric of the pink species formed by reaction of metformin.HCl(50 mM) in alkaline medium(50 mM) followed by the reaction with Cu(II) ion (500 µg.$ml^{-1}$).(distilled water as a reference).***

### 3.3. Study of the optimum parameter for the determination of Metformin.HCl via FIA

#### 3.3.1. Chemicals parameters

It quite necessary to study their effect inorder to established the optimum concentration for the material used to form the

complex for MTF-$OH^-$-Cu(II) to obtain maximam absorbance for the colored complex, it was noticed that at nil concentration for any individual of the chemicals used for the reaction the absorbance will be zero in another way it means incomplete formation of the colored complex.

### 3.3.1.1. Copper (II) ion concentration effect

A variable Cu(II) ion concentration ranging from 0.0-350 $\mu g.ml^{-1}$ was studied, It was noticed an increase in the absorbance of the complex with increasing Cu(II) ion concentration, while at higher concentration above 225 $\mu g.ml^{-1}$ there was a decrease in the absorbance of the complex; this might be due to the precipitation of Cu(II) ion as $Cu(OH)_2$ causing a decrease in the height of the response peak and its irregularities as shown in Fig.(4). On this basis the 225$\mu g.ml^{-1}$ was the optimum Cu(II) ion that satisfies the MTF-$OH^-$-Cu(II).

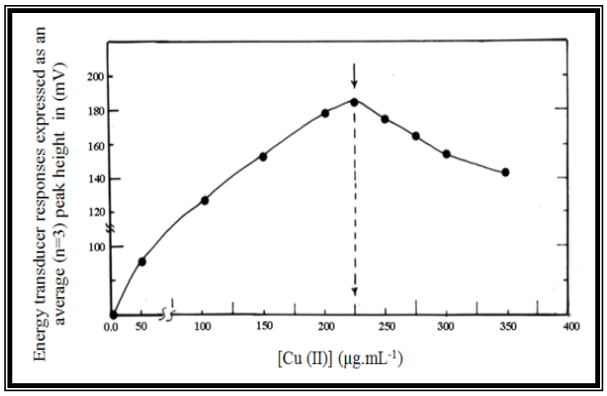



***Fig.(4) Variation of energy transducer response expressed as average peak height in (mV) versus concentration of Cu (II) expressed in ($\mu g.ml^{-1}$).***

### 3.3.1.2.Sodium hydroxide solution concentration effect

A serious of sodium hydroxide solution were prepared ranging from 0.0-50 mM, It was noticed that the increase in the base concentration($OH^-$) up to 8mM gave a regular with a suitable peak height comparing with a lower concentration of 8mM the responses were of low sensitivities(low response). This might be due to not reaching the optimum level of the best concentration to the necessary level; while an increase on the ($OH^-$) above 8 mM, it was noticed that were no significant differences on the height of the responses (Fig.(5)), this might be attributed to the formation of the precipitate and does not aid in the determination process as it takes a longer time to discharge and evacuate the complex or the formed precipitate in the measuring cell. On this basis 8 mM was chosen.

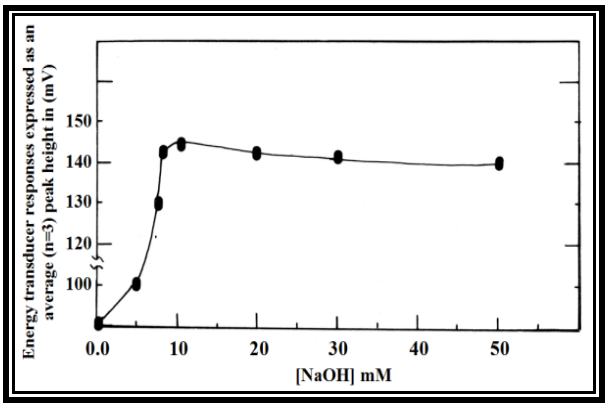



***Fig.(5) Variation in energy transducer response in (mV) (peak height n=3) versus sodium hydroxide solution concentration.***

### 3.3.2. Physical parameters

### 3.3.2.1. Flow rate

Using optimum variable of chemicals for the determination of MTF.HCl, the flow rate for three lines was investigated. As is observed in Fig.(6A), an increase in the absorbance with increase of flow rate reaching up to 1.1 $ml.min^{-1}$, this might be attributed that the physical variable has realy no such significant effect, important must of it the dilution of the sample segment due to dispersion region surrounding the central part of the segment and the central dispersion due to diffusion and convection; while an increase in the flow rate above 1.1 $ml.min^{-1}$ led to a decrease in the responses, this might be due to incompletion of the reaction to form the complex or the unavailability of enough time for the absorbance measurement before its departure of the measuring cell at a short time. In addition the time of the arrival of the sample segment to the measuring cell decrease with the increase of the flow rate (Fig.(6B));also there is a decrease in the width of the response ($\Delta t_B$) as shown in Fig.(6C).

On this basis the flow rate for the three lines were chosen as 1.1 $ml.min^{-1}$.

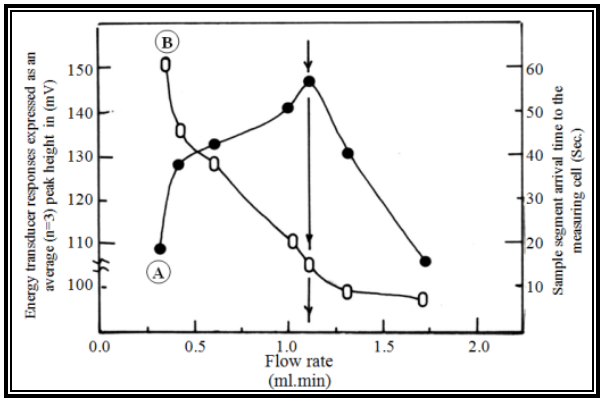
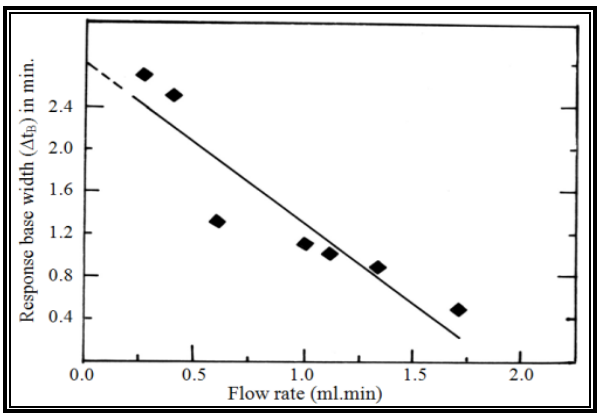



***Fig.(6) Variation in flow rate against:***

A- ***transducer response expressed as peak height in mV.***

B- ***Energy Arrival time of sample segment to the measuring cell.***




C- **Decrease of ΔtB (response base width) with the increase of flow rate.**

### 3.3.2.2. Injected sample volume

Using optimum parameters for the reaction MTF-$OH^-$-Cu(II) with a variable sample loop volume ranging from 10-80µL. From the results obtained Table (2), it can be noticed the increase in the response height and an increase in its base width with an increase with the injected sample volume; while at a volume larger than 40 µL has not significant differences as in Fig.(7) and for the purpose to compromise with the height of the response and adjustment in the cost in the consumption of the chemicals; 40µL was chosen as the best injected sample volume whereby a smooth profile with a sample maxima.

***Table (2)***

***Effect of the variation of injected sample volume on the absorbance at a selected Metformin concentration of 40mM.***

| *Injectd sample volume (µL)* | *Absorbance $y_i$ (mV)* | *Average $\bar{y}_i$ (mV)* | *ΔtB (min)* | *Arrival time to the measuring cell (sec)* |
|---|---|---|---|---|
| **10** | **72,84,84** | **80** | **0.6** | **6** |
| **20** | **106,106,107** | **106.33** | **0.75** | **9** |
| **30** | **130,130,130.4** | **130.13** | **0.85** | **12** |
| **40** | **145.6, 145.6, 145.6** | **145.6** | **1.0** | **15** |
| **50** | **148, 148, 148** | **148** | **1.5** | **18** |
| **80** | **150, 150, 150** | **150** | **1.75** | **28** |

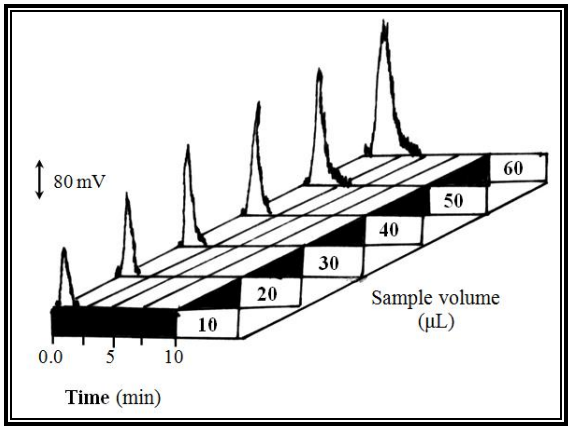



***Fig.(7) Effect of the Variation of sample volume on the peak height response for the determination of MTF injected on carrier stream.***

### 3.3.2.3. Allowed permissible time

Allowed permissible time for the sample to be injected via the carrier stream was studied. As is observed in Fig.(8), an increase in the response with increasing the allowed permissible time for the sample to injected up to 20 sec, followed by a constancy in the response with an increase in the width of the base of the response it can be inference that an increase in the injection time above 20 sec causes to the distraction of the flow as a result of elongated of period of leaving of injected sample volume in the valve in the "Injection" mode which leads to the slow movement of the colored segment in the measuring flow cell for a longer period of time; on this basis 20seconds was chosen which causing to have a suitable peak height and a regular profile of the response.

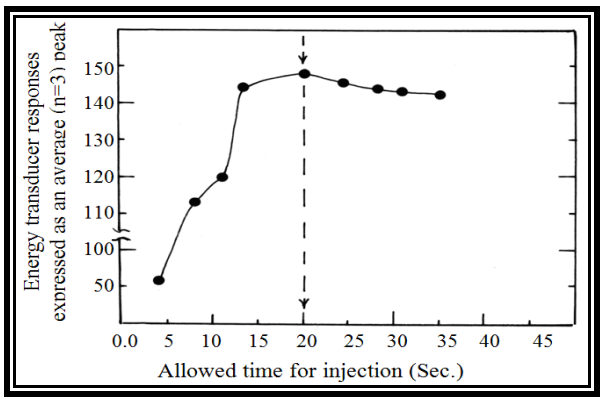



***Fig.(8) Variation of energy transducer response expressed as an average peak height in (mV) versus time of injection for sample with (40µL).***

### 3.3.2. Study of the variation of Metformin concentration on the response of the complex formed

A serious of metformin solution having the concentration of 0.0-200 mM using 40 µL as an injected sample volume with all the chemical and physical variable fixed at its optimum parameters. Using simple linear robustic equation of degree one at 95% confidence interval as shown in Fig.(9); all these results of the linear regression analysis was summarized in Table (3). The limit of detection(defined as the analyte concentration giving a signal equal to blank signal, ($y_B$), plus three standard deviations of the blank, ($S_B$)[15] was determined; using successive gradual dilution of the minimum concentration of metformin drug that was used in the calibration graph which was 0.9 mM a limit of detection of 662 ng/injected sample volume.

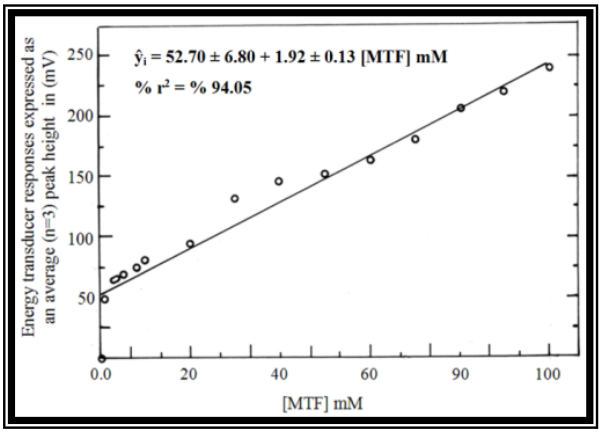



***Fig.(9) Linear calibration graph for the energy transducer response with metformin concentration expressed in mM.***

***Table (3)***
***Summary of linear regression for the variation of signal response with metformin concentration using first degree equation of known form y=a+bx.***

| Concentration mM | Linear range mM | Straight line equation $\hat{y}_{mv}=a\pm s_a t+b\pm s_b t$ [x] | Correlation coefficient (r) | Percenta-ge linearity ($r^2$%) | calculated t-value $\frac{\lvert r\rvert\sqrt{n^*-2}}{\sqrt{1-r^2}}$ | t-value tabulated at 95% confidence interval |
|---|---|---|---|---|---|---|
| (0.0-200) | (0.0 - 100) | ŷ=52.70±6.80 +1.92±0.13 [x]mM | 0.9697 | 94.05 | 14.33 | 2.16 |

***n*=15***
***[x]=Concentration of drug in mM.***
***y= Energy transducer response expressed as average peak height in (mV); ŷ=Predict of energy transducer response expressed as average peak heightof the linear Robustic equation of the form: y=a+bx.***

### 3.3.3. Application

The used microphotometer throughout this work was put into a test for its efficiency of the measurement in three different metformin drug in three different pharmaceutical preparation from different origin of supplier. Metformin.HCl in each pharmaceutical drug was determined using Continuous Flow Injection Analysis (C.F.I.A) and the result was expressed in a direct calibration graph and Table (4) summarizes the results obtained at confidence 95% successively and it was noticed that the recovery ranges from 89.17 to 96.66 %.

***Table (4)***
***Determination of metformin at different manufactures of pharmaceutical drugs in direct calibration graph by using MTF-OH$^-$-Cu(II) system.***

| *Sample number* | *Confidence interval of concentration of tablets for n=∞ of tablets at 95%* $\bar{w}\pm1.96\frac{sn-1}{\sqrt{n}}$ *& confidence interval at 99%* $\bar{w}\pm2.58\frac{sn-1}{\sqrt{n}}$ | *Confidence interval of concentration of active material for n=∞ of tablets at 95%* $\bar{w}\pm1.96\frac{sn-1}{\sqrt{n}}$ *& confidence interval at 99%* $\bar{w}\pm2.58\frac{sn-1}{\sqrt{n}}$ | *Practical weight (mg)* | *Recovery %* |
|---|---|---|---|---|
| 1 | 0.75±0.0051<br>0.751± 0.0068 | 500±3.39<br>500±4.52 | 443.06 | 89.17 |
| 2 | 0.5508±0.0012<br>0.5508±0.0016 | 500±1.089<br>500±1.452 | 455.48 | 91.67 |
| 3 | 0.5289±0.0038<br>0.5289±0.0051 | 500±3.592<br>500±4.821 | 480.32 | 96.66 |

Since the active metformin material is mixed through the process of tablet formation by the addition of additives therefore this leads us to use the standard addition method, which might overcome the difficulty of having different matrices; Table (5) tabulated the results obtained which shows percentage recovery of 90.02 up to 101.49.

***Table (5)***
***Determination of Metformin in different pharmaceutical drugs by the standard addition curve for the complex MTF-OH--Cu(II) formation.***

| Sample number | Practical weight (Theoritical weight 500mg) | Intercept (a) & Slop (b) | Correlation coefficient r Percentage linearity ($r^2$) | Calculated t-valu $\frac{\lvert r\rvert\sqrt{n^{*}-2}}{\sqrt{1-r^2}}$ | Tabulated t-value at 95% confidence interval | Recovery % |
|---|---|---|---|---|---|---|
| 1 | 450.11 | (79.68)<br>1.14 | 0.9974<br>(%99.49) | 24.25 | 3.18<< | 90.02 |
| 2 | 475.15 | (82.32)<br>1.25 | 0.9977<br>(%99.54) | 25.54 | | 95.03 |
| 3 | 507.49 | (83.76)<br>1.29 | 0.9989<br>(99.79) | 38.53 | | 101.49 |

Metformin. HCl was determined by the conventional classical spectrophotometric measurement it can be seen from table 6 and extended of linearity of linear regression equation as percentage linearity at 97.48%, Table (6) also shows practically measured concentration in the three pharmaceutical drugs.

***Table (6)***
***Determination of Metformin in different pharmaceutical drugs using the conventional method***

| Concentration range (mM) | Linear range (mM) | $\hat{y}=a\pm sat+b\pm sbt[x]$ | Correlation coefficient r Percentage linearity ($r^2$) | Calculated t-valu $\frac{\lvert r\rvert\sqrt{n^{*}-2}}{\sqrt{1-r^2}}$ | Tabulated t-value at 95% confidence interval | Sample number | Found value (mM) | Recovery % |
|---|---|---|---|---|---|---|---|---|
| 0.9-380 | 5-240 | $\hat{y}=0.088\pm0.0056+0.00087\pm4.94\times10^{-5}[x]$mM | 0.98731<br>% 97.48 | 17.61 | 2.306 | 1 | 26.5 | 88.33 |
| | | | | | | 2 | 27.0 | 90.01 |
| | | | | | | 3 | 28.5 | 95.00 |

Using statistical chemometric treatment and the results of both methods were tested using paired t-test. Table (7) shows that there are no significant differences between the newly developed method and the conventional

traditional spectrophotometric method at confidence interval 95%, as the calculated value is less than the tabulated value; therefore the newly developed method can be regarded or used as an alternative method for the determination of metformin. Also the speed of analysis, small consumption chemicals and higher sensitivity which lead the method to follow the small concentration pattern.

***Table (7)***
***Paired t-test for the comparison of the spectrophotometric method with continuous flow injection method of analysis adopted through this work.***

| Sample number | Practical content (mg) | | (d) (mg) | $\bar{X}_d$ | σn-1 | Paired t-test $\frac{\bar{X}_d\sqrt{n^*}}{\sigma_{n-1}}$ | Tabulated t-value at 95% confidence interval |
|---|---|---|---|---|---|---|---|
| | UV-Vis | SP. - FIA | | | | | |
| 1 | 441.67 | 443.06 | 1.39 | 4.04 | 2.30 | 3.042 < 4.303 | |
| 2 | 450.05 | 455.48 | 5.43 | | | | |
| 3 | 475.00 | 480.32 | 5.32 | | | | |

## Conclusion

The work presented in this thesis; present a sample of completeness between the preparation for a complex species from the chelate of one of the most important drug, which is metformin regarding these drug as an inorganic chelate reagent for copper ion via the formation of the color species complex , then using these approach for the formation of the complex through spectrophtometric determination of drug without using of chemical reagent with addition of establishing a complex microphotometer (homemade) for the online and continuous analysis for one of the most important drug. The established method were subjected for the comparison with use of a worldwide used spectrophotometer (UV-Visible Spectrometer (Cary-Varian El 04103410)) for comparison. The homemade system which was built in the laboratory was at a level or even it exceed that level the absorbance of 2, which is the logarithm of two that means complete absorbance of the incident light, which is the maximum limit for the spectrophotometer determination above this limit; deviation can be expected, while the newly design system can exceed that the limit as it contain a completely different approach regarding the source, cell type, its geometry, the detector used the amplification of the energy transducer response. Expressing the response digitally or plotting on x-t recorder or even both can be represented simultaneously. All the results were subjected to data treatments, which show that there were no significant differences between well recognized instrument and the homemade unit.

## References


[1] The Indian Pharamcopoeia, New Delhi: The controller of publications, 4th Ed., Vol.1; 469; 1996.

[2] Budavari, S.; editotrs. In, The Merck Index. 13th Ed., Merck & Co. Inc. 998; 2001.

[3] Bailey, C.J. "Biguanides and NIDDM"; Diabetes Care 15, pp 755-772, 1992.

[4] Dorman,T.; "Double-blind evaluation of efficacy and tolerability to metformin in NIDDM"; Diabetes care 14, pp 342-344, 1991.

[5] Siest,G.; Roos,F.; Gabou,J.J.; "Dosage du N,N-dimethyl biguanide(glycophage) par le diacetyl en milieu alcalin"; Bull. Soc. Pharm. Nancy 58, pp 29-38, 1963.

[6] Pignard, P.; "Spectrophotometric determine-ation of N, N-dimethyl biguanide in blood and urine"; Ann. Biol. Clin 20, pp 325-333, 1962.

[7] British Pharmacopoeia, Vol II, Cambridge University press, Cambridge, 1988.

[8] Garrett,E.R. ;Tsau,J. "Application of ion-pair methods to drug extraction from biological fluids"; J. Pharm.Sci. 61, pp1404-1410, 1972.

[9] Mubeen,G.; Noor, "Spectrophotometric method for Analysis of metformin hydrochloride", Indian J. Pharms. Sci. 71, pp 100-102, 2009.

[10] Alkalay, D.; Volk, J.; Bartlett, M.F.; "Conversion of biguanides into substituted s-triazines assayable by GC or mass fragmentography"; J. Pharm. Sci. 65, pp 525-529, 1976.

[11] Sane, R.T.; Banavaliker, V.J.; Bhate, V.R.; Nayak, V.G, "Gas chromatographic determination of metformin hydrochloride from pharmaceutical preparations"; Ind. Drugs 26, pp 647-648, 1989.
[12] Vasudevan, M.; Ravi, J.; Ravishankar, S; Suresh, B.; "Ion-pair liquid chromatography technique for the determination of metformine in its multi component dosage forms"; J. Pharm. Biomed. Anal. 25, pp 77-84, 2001.
[13] Calatyud, J.M. "Flow Injection Analysis of Pharmacentical", in: Automation in the Laboratory, Taylor & Francis, London, 898; 1996.
[14] Shakir, I.M.A.; Turkie, N.S.; Patent, No.001667, presented to ministry of Planning, 2011.
[15] Guide, A.B."Practical Statistics for the Analytical Scientist", 1st Ed. Government Chemistry, Teddington, Middlesex. UK., 97 1997.

**الخلاصة**

تم تطوير طريقة طيفية حديثة أستخدمت في موضوع البحث لتقدير عقار الميتفورمين من خلال تكوين معقد للدواء مع آيون النحاس الثنائي. تم قياس طيف المعقد الملون عند 530 نم. تم وصف مفاضلة الظروف الكيميائية والفيزيائية لنظام تقدير الميتفورمين بتكوين معقد مع آيون النحاس الثنائي في الوسط القاعدي وتم التوصل الى مدى خطي للتقدير بحدود 94.04% للمدى من 0.0−100 مللي مول/ لتر مع حد كشف 662 نغم ثم تم استخدام النظام والطريقة المطورة لتحليل العديد من التحضيرات الدوائية التجارية ومن ثم استخدام معالجات رياضية مستندة على إختبار المزدوج-t والذي أظهر بأنه لايوجد فرق جوهري للطريقة المستخدمة وإمكانية إستخدامها كطريقة تحليلية بديلة لتقدير عقار الميتفورمين. إعتمد مشروع البحث على إجراء كافة التفاعلات والتقديرات باستخدام التحليل بالحقن الجرياني المستمر.