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SPECTROPHOTOMETRY DETERMINATION OF METFORMIN BY USING A 9-PHYNEL 2.3.7 HYDROXY -6- FLUORINE AS REAGENT

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ABSTRACT

A new spectrophotometric method for determination of Metformin depending on the reaction between the Metformin and 9,phenyl 2,3,7, hydroxyl 6- fluorine reagent . Simultaneous determination of Metformin in concentration interval of (10–50µg.ml^{-1}) by measuring the amplitude of peak-to-base line, zero cross at certain wavelengths and the area under peak at selected spectrum intervals. The methods showed reasonable precision and accuracy and have been applied to determine Metformin in four different pharmaceutical preparations.

KEYWORDS: Spectrophotometry, Metformin

INTRODUCTION

Metformin is an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function.[1] Its use in gestational diabetes has been limited by safety concerns. It is also used in the treatment of polycystic ovary syndrome, and has been investigated for other diseases where insulin resistance may be an important factor. Metformin works by suppressing glucose production by the liver.[2][3]

Metformin was first described in the scientific literature in 1922, by Emil Werner and James Bell, as a product in the synthesis ofN,N-dimethylguanidine.[4] In 1929, Slotta and Tschesche discovered its sugar-lowering action in rabbits, noting it was the most potent of the biguanide analogs they studied.[5] This result was completely forgotten, as other guanidineanalogs, such as the synthalins, took over, and were themselves soon overshadowed by insulin.[6]

Interest in metformin, however, picked up at the end of the 1940s. In 1950, metformin, unlike some other similar compounds, was found not to decrease blood pressure and heart rate in animals.[7] That same year, a prominent Philippine physician, EusebioY.Garcia,[8] used metformin (he named it Fluamine) to treat influenza; he noted the drug "lowered the blood sugar to minimum physiological limit" and was not toxic. Garcia also believemetformin to havebacteriostatic, antiviral, antimalarial, antipyretic, and analgesic actions.[9] In a series of articles in 1954, Polish pharmacologist JanuszSupniewski[10] was unable to confirm most of these effects, including lowered blood sugar; he did, however, observe some antiviral effects in humans.[11][12]

Limited evidence suggests metformin may prevent the cardiovascular and possibly the cancer complications of diabetes.[13--15] It helps reduce LDL cholesterol and triglyceride levels and is not associated with weight gain; in some people, it promotes weight loss.[15--19] Metformin is one of only two oral antidiabetics in the World Health Organization Model List of Essential Medicines (the other being glibenclamide).[16--19]

The usual synthesis of metformin, originally described in 1922 and reproduced in multiple later patents andpublications, involves the reactionof dimethylaminehydrochlorideand 2-cyanoguanidine (dicyandiamide) with heating. [20]

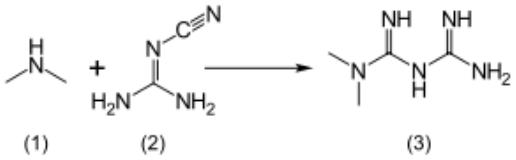

Figure 1: The Reaction of Dimethylaminehydrochloride and 2-Cyanoguanidine (Dicyandiamide) with Heating

According to the procedure described in the 1975 Aron patent,[21] and the Pharmaceutical Manufacturing Encyclopedia,[22] equimolar amounts of dimethylamine and 2-cyanoguanidine are dissolved in toluene with cooling to make a concentrated solution, and an equimolar amount of hydrogen chloride is slowly added. The mixture begins to boil on its own, and after cooling, metformin hydrochloride precipitates with a 96% yield.

Metformin has acid dissociation constant values (pKa) of 2.8 and 11.5, so exists very largely as the hydrophilic cationic species at physiological pH values. The metformin pKa values make metformin a stronger base than most other basic drugs with less than 0.01% unionized in blood. Furthermore, the lipid solubility of the unionized species is slight as shown by its low logP value [log(10) of the distribution coefficient of the unionized form between octanol and water] of -1.43. These chemical parameters indicate low lipophilicityand, consequently, rapid passive diffusion of metformin through cell membranes is unlikely. The logP of metformin is less than that of phenformin (-0.84) because two methyl substituents on metformin impart lesser lipophilicity than the larger phenylethyl side chain in phenformin. More lipophilic derivatives of metformin are presently being investigated with the aim of producing prodrugs with better oral absorption than metformin itself.[23]

Figure 2: Generic Metformin 500-Mg Tablets, as Sold in the United Kingdom

Metformin is sold under several trade names, including Glucophage XR, Carbophage SR, Riomet, Fortamet, Glumetza, Obimet, Gluformin, Dianben, Diabex, Diaformin, Siofor, and Metfogamma.

Liquid metformin is sold under the name Riomet in India. Each 5 ml of Riomet is equivalent to the 500-mg tablet form of metformin.[24]

Metformin IR (immediate release) is available in 500, 850, and 1000-mg tablets. All of these are now available as generic drugs in the U.S.

Metformin SR (slow release) or XR (extended release) was introduced in 2004. It is available in 500, 750, and 1000-mg strengths, mainly to counteract the most common gastrointestinal side effects, as well as to increase compliance by reducingpill burden. No difference in effectiveness exists between the two preparations.

The purpose of this work is to determine Metformin using reaction with 9,phenyl 2,3,7, hydroxyl 6- fluorine reagent spectrophotometry and to demonstrate that these methods can be very useful tools for determining Metformin in mixture, without tedious and time consuming separation procedures.

Experimental

Apparatus

A Shimadzu UV1601 double beam UV-VIS spectrophotometer was loaded with Shimadzu UVProb Version 1.10 software to record the spectra and perform subsequent calculations of their derivatives.

The spectrophometric measurements were made at wavelength range 200-350 nm using 1 cm quartz matched cells. The spectra were recorded with a fast scan speed, sampling interval=1.0 and slit width=2.0 nm.

Reagents

Metformin Standard Solution :(10-50 μg.ml^{-1}): 0.01 g of Metformin (obtained from the state company for drug industries and medical appliance (S.D.I.), Samara-Iraq) is dissolved in 100 ml in a volumetric flask with doubled distilled water. Working solutions were freshly prepared by subsequent dilutions.

Analysis of capsule: The content of 10 capsules were mixed well and a certain portion of the fine powder was accurately weight to give an equivalent to 0.01g of Metformin, and dissolved in 1 ml 0.1 M sodium hydroxide solution. The resulted solution was diluted to 100 ml with double distilled water in a volumetric flask. The solution was filtered by using Whatmann filter paper No. 40 to avoid any suspended or un dissolved material before use.

Procedure

Individual Determination of Metformin

In 10 ml calibrated flask, transfer aliquots of Metformin solutions expected to contain (10-50 μg) and dilute to the mark with doubled distilled water. The absorption spectra were recorded and show absorption maxima at 214 nm for Metformin.

Determinations were made by measuring the druge with the reagent first of their spectra at certain given wavelengths. The concentration of Metformin could be determined respectively.

Simultaneous Determination of Metformin:

The content of different amounts (10 – 50 μg) of Metformin, were diluted with doubled distilled water. The absorption spectra were recorded against blank (prepared by the same manner as test solution but without Metformin) .the concentration of Metformin could be determined.

RESULTS AND DISCUSSIONS

Absorption spectra

The absorption spectra of Metformin and for their mixture were recorded. Figure 3 shows the absorption spectrum of Metformin solution (20 µg. ml^{-1}) with two maxima at wavelength 260 nm, curve show the absorption spectrum of with maximum wavelength of absorption at 280nm. The total spectrum of mixture of (200 µg of each per 10 ml) is shown in curve c with λmax (280 nm) between the absorption maxima of the two components.

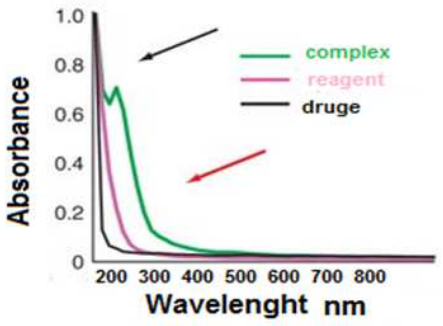

Figure 3: Absorpstion Spectra of (A) 20 Mg.Ml^{-1} of Metformin, (B) 20 Mg.Ml^{-1} of 9 Phenyl 2, 3, 7, Hydroxyl 6- Fluorine Reagent, (C) Complex

In the present work, graphically (peak-to-base line), technique in addition to peak area were used to deal with complex spectra to carry out the measurements. In fact that all these techniques show good proportionality to Metformin concentrations in their mixtures.

Calibration Graph and Statistical Analysis

The analytical characteristics and most statistical data for the proposed method are given in Table 1. Under optimum conditions, linear calibration graphs were obtained in the range of (10 – 50 µg.ml^{-1}) with correlation coefficient values ranging between (0.9995 – 0.9842) and detection limits values in the range of (4.2-0.21µg.ml^{-1}) for different techniques of measurements.

Accuracy and Precision

Under the optimum conditions, the accuracy and precision of the proposed method were checked. Table 2 shows the values of relative error percent and relative standard deviation percent for two different level of concentration of Metformin.

Application of the Methods

The proposed method were successfully applied for direct determination of Metformin in three different drugs. The results obtained are presented in Table 3, and are in quite agreement with the spiked values. On the other hand, Metformin has also been successfully determined in three different pharmaceutical preparations by the proposed method. The results are shown in table 3

Table 1: Statistical Analysis of the Determination of Metformin

Compound	Mode of Calculation	Λ (Nm)	Regression Equation	R	D. L. $Mg.Ml^{-1}$
Metformin	Peak area	202-224	Y=0.1105-0.01181 X	-0.8921	6.20
	Peak area	224-262	Y=-0.0135-0.00774 X	-0.9987	0.56
	Peak area	224-237	Y=0.0001+0.00047 X	0.9957	0.80
	Peak area	237-259	Y=0.0055+0.00057 X	0.9991	2.00
	Peak to base line	236	Y=0.0645+0.000485 X	0.9987	0.21
	Peak to base line	244.6	Y=0.0004+(3.87e-5) X	0.9993	0.27

Table 2: Precision and Accuracy of the Methods

Compound	Method of Analysis	Taken ($Mg.Ml^{-1}$)	Fond * ($Mg.Ml^{-1}$)	Relative Error %	Relative Standard Deviation %
Metformin	First order (peak-to-base line) at 236nm	10	10.213	+2.130	2.130
		60	60.460	+0.766	0.299
	Second order (peak-to-base line) at 244.6 nm	10	10.197	+1.970	0.568
		60	60.343	+0.572	0.165

* Average of four determinations.

Table 3: Results for Analysis of Metformin in Four Pharmaceutical Formulation Samples

Pharmaceutical Preparation	Labeled Amount Mg/Tablet	Found Amount Mg/Tablet		
		Mean Value*	RSD%	E%
Metformin S. D. I. - Iraq	250	244.53	0.74	-2.18
	500	493.66	0.42	-1.27
	250	245.90	0.64	-1.64
	500	494.10	0.40	-1.18
Metformin MICRO LABS Ltd India	250	242.67	0.98	-2.93
	500	491.00	0.44	-1.80
	250	242.79	0.59	-2.88
	500	492.81	0.50	-1.44
Metformin APKES Ajanta-Pharma Ltd	250	244.10	1.98	-2.15
	500	489.61	0.93	-2.08
	250	245.01	0.65	-1.99
	500	489.99	0.41	-2.00

* Average of three determinations.

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APPENDICES

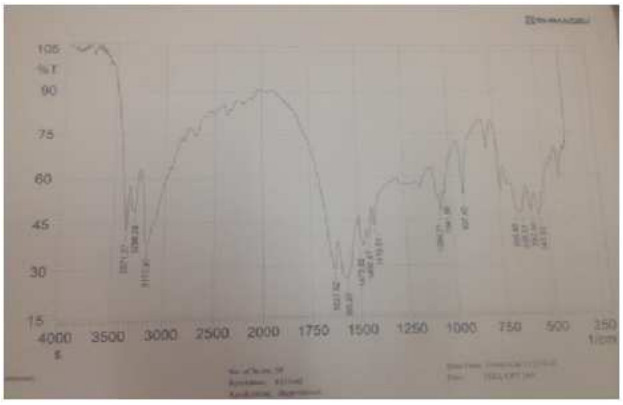

Figure 4: FTIR Spectra of (a) 20 µg.ml^{-1} of Metformin

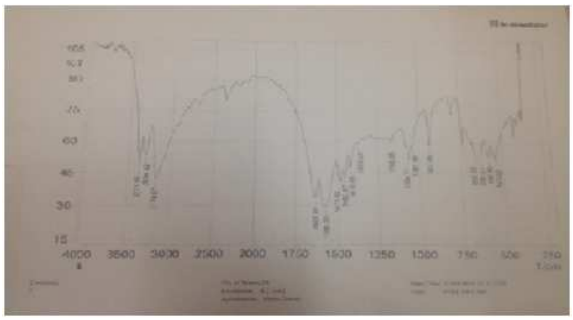

Figure 5: FTIR Spectra Spectra of (B) 20 Mg.Ml^{-1} of 9 Phenyl 2, 3, 7, Hydroxyl 6- Fluorine Reagent

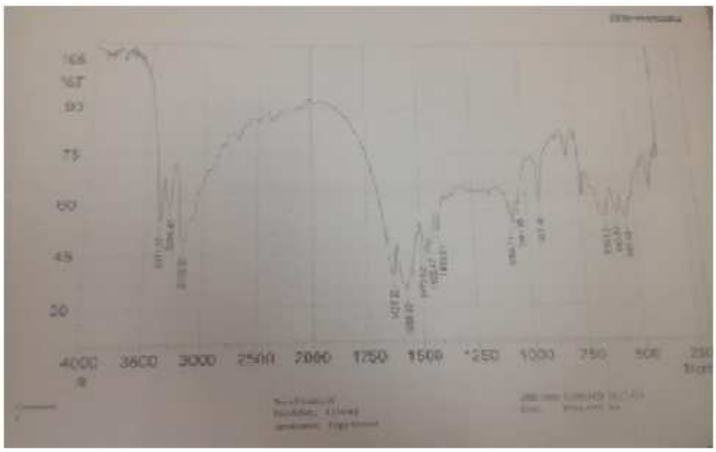

Figure 6: FTIR Spectra of (C) Complex