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ATOMIC ABSORPTION AND ATOMIC EMISSION SPECTROMETRY FOR THE MICRO-DETERMINATION OF METFORMIN EMPLOYED IN CONTROL OF BLOOD SUGAR LEVEL IN PHARMACEUTICAL FORMULATIONS AND URINE SAMPLES

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ABSTRACT

Ion-associate complexes of metformin hydrochloride drug employed in control of blood sugar level with (manganese(II), zinc(II) and cadmium(II)) thiocyanates and ammonium reineckate are precipitated. The solubility of the solid complexes at the recommended optimum conditions of pH and ionic strength values have been studied. Saturated solutions of each ion associate at different temperatures under the optimum precipitation conditions were prepared and the metal ion contents in the supernatant were determined. A new accurate and precise method based on direct coupled plasma atomic emission and atomic absorption spectrometry for the determination of metformin (0.33-36.42 µg/ml) in pure solution, pharmaceutical Formulations and in urine samples of diabetic patients type-2 in Taif Area, Saudia Arabia is given.

KEYWORDS: Ion associate complexes, Pharmaceutical Analysis, Atomic Emission and Atomic Absorption Spectrometry.

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INTRODUCTION

Metformin hydrochloride;(Mf), (glucophage)[1], chemically is 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 primary by inhibiting hepatic gluconeogenesis and glucogenolysis[2] and seems to ameliorate hyperglycemia by improving peripheral 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 reveres fatty liver diseases[3] . There is some evidence that early treatment with metformin is associated with reduced cardiovascular morbidity and total mortality in newly diagnosed type 2 diabetic patients.[4] However, the data come from a small subgroup of a much larger trial. In contrast, despite multiple trials of intensive glucose control using a variety of glucose-lowering strategies, there is a paucity of data to support specific advantages with other agents on cardiovascular outcomes.[5–7] In the original UK Prospective Diabetes Study (UKPDS), 342 overweight patients with newly diagnosed diabetes were randomly assigned to metformin therapy.[8] After a median period of 10 years, this subgroup experienced a 39% (*P* = 0.010) risk reduction for myocardial infarction and a 36% reduction for total mortality (*P* = 0.011) compared with conventional diet treatment. Similar benefits were not observed in those randomly assigned to sulfonylurea or insulin. In an 8.5-year post trial monitoring study, after participants no longer were randomly assigned to their treatments, individuals originally assigned to metformin (*n* = 279) continued to demonstrate a reduced risk for both myocardial infarction (relative risk 33%, *P* = 0.005) and total mortality (relative risk 27%, *P* = 0.002).[9] The latter results are even more impressive when one considers that HbA1c levels in all initially randomly assigned groups had converged within 1 year of follow-up. 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[10-21]. Another different methods for the determination of metformin have been described , such as conductometric titration[22], flow-injection chemiluminescence [23-25], capillary electrophoresis[26], ion- selective electrode[27] and adsorptive catalytic square-wave voltammetry.[28] 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.[29] The colorimetric methods include charge transfer complex with iodine in acetonitrile medium [30] , reaction of metformin with Cu^{+2} in basic cyclohexyl amine medium[31] and the reaction with ninhydrin to form a violet colored complex,[32] spectrophotometric method using multi variate technique[33] and other spectrophotometric methods. [34-36] However all of these methods suffered from several disadvantages including use of complex extraction procedures which were tedious and time consuming. The importance of this drug has prompted the development of many methods for its determination. The use of simpler, faster, less expensive and sensitive method is desirable. The proposed method is simple and applicable as well as for routine analysis of metformin hydrochloride in tablets and urine samples of diabetic patients type-2 in Taif Area, Saudia Arabia.

MATERIALS AND METHODS

Double-distilled water and analytical grade reagents were used to prepare all solutions. Metformin hydrochloride (Gulf Phrmaceutical Industries,RasAlKhaimah,U.A.E.), mmonium reineckate, manganese chloride and zinc acetate were Aldrich products, cadmium nitrate (BDH), Glucophage tablets, containing 1000 mg Mf HCl per tablet were obtained from (Merck Sante S. A. S., France), Glucophage XR tablets, containing 750 mg Mf HCl per tablet were obtained from (Merck Serono, KGaA, Germany) and Diolan tablets, containing 500 mg Mf HCl per tablet were obtained from (Gulf Phrmaceutical Industries, Ras Al Khaimah, U.A.E.).

APPARATUS

The pH of solutions was measured using an Orion (Cambridge, MA, USA) digital pH meter. Direct coupled plasma atomic emission measurements were carried out using ICPE-9000 Shimadzu plasma atomic emission spectrometer and atomic absorption measurements were made on AA-6650 Shimadzu atomic absorption spectrophotometer. Conductimetric measurements were carried out using conductivity measuring bridge type M.C.3 model EBB/10 (K_{cell} = 1);[Chertsey, Surry, England]. The IR absorption spectra were obtained by applying the KBr disk technique using a PYE UNICAM SP – 300 infrared spectrometer.

PREPARATION OF ION ASSOCIATES

The ion associates were prepared by mixing solutions containing 1 x 10^{-3} mol of Mn(II), Cd(II), or Zn(II) with a solution containing 4 x 10^{-3} mol of potassium thiocyanate and the requisite amount of Mf HCl. Ammonium reineckate 1 x 10^{-3} mol of the solution was mixed with the calculated amount of Mf HCl. The precipitates obtained were filtered, thoroughly washed with distilled water, and dried at room temperature. They were subjected to elemental microanalysis, infrared spectroscopy, nuclear magnetic resonance and determination of the metal content.

EFFECT OF pH ON THE SOLUBILITY OF ION ASSOCIATES

The choice of a suitable pH value at which the ion associates exhibit the lowest solubilities and the effect of pH on the degree of completeness of ion-associate formation were studied as follows: the solid ion associates were added to form saturated solutions in a series of solutions of different pH values ranging from 1 to 10; the pH value was adjusted with 0.1 *M* HC1 or 0.1 *M* NaOH. The solutions were shaken for 4-6 h and left to stand for a week to attain a stable equilibrium. Then the saturated solution is filtered in a dry beaker (rejecting the first few milliliters of filtrate). One milliliter of the filtrate is transferred into a 100-ml measuring flask containing 1 ml of concentrated $HN0_3$ and the volume is filled to the mark with distilled water. The equilibrium concentration of the metal ion present in the form of soluble inorganic complex ion is measured using DCP-AES, and hence the solubility of the precipitate is evaluated, from which the solubility products of the ion associates were calculated.

EFFECT OF IONIC STRENGTH ON THE SOLUBILITY OF ION ASSOCIATES

A series of saturated solutions of the ion associate adjusted to the optimum pH value and having different ionic strength (0.1-1.0) was prepared using NaCl as the electrolyte. The same procedures as those used in the determination of the effect of pH have been followed to determine the optimum ionic strength values at which ion associates have the lowest solubilities.

PREPARATION OF THE STANDARD SOLUTIONS

Standard solutions of divalent cadmium, chromium and zinc are prepared by weighing 1.0 g of a high-purity sample (cadmium shot, manganese shot, chromium shot and zinc metal, respectively), transferring it to a 1-liter measuring flask and then adding 50 ml of concentrated HNO_3. After dissolution the solution is diluted to 1 liter with deionized water. The 1000-ppm solution is stored in a plastic bottle which has been presoaked in dilute $HN0_3$. The solution is stable for approximately one year. Standard solution of iron was obtained from Aldrich.

CALIBRATION OF ATOMIC SPECTROMETRY INSTRUMENTS

Under the recommended conditions, calibration graphs were constructed of aqueous standards of cadmium(II), chromium(III), zinc(II) and Mn(II) in 1 M $HN0_3$ by performing triplicate measurements using solutions containing 0, 10, 20, and 50 ppm analyte concentrations as previously reported.[37-39] The calibration graphs are straight lines passing through the origin.The different parameters used for the measurement of cadmium(II), chromium(III), zinc(II) and Mn(II) are listed in Table 1. The atomic absorption spectrometry was calibrated as in the previously reported work.[40 – 42]

CONDUCTOMETRIC MEASUREMENTS

The stoichiometry of the ion associates was elucidated by conductimetric titration of Mf

HCL with the metal complex solutions

ANALYTICAL DETERMINATION OF MITFORMIN HCl IN AQUEOUS SOLUTIONS

liquots (0.05 - 5.5 ml) of 0.001 *M* Mf cit solution are quantitatively transferred into 25-ml measuring flasks. To each flask 1.0 ml of 0.01 *M* standard solution of (Cd(II), Mn(II) or Zn(II)) thiocyanate, or ammonium reineckate is added and the flask is filled to the mark with the recommended buffer solution of the optimum pH and ionic strength values. The solutions are shaken well and left to stand for 15 min and then filtered through Whatman P/S paper (12.5 cm), and the equilibrium metal ion concentration in the filtrate is determined using DCP-AES. The metal ion consumed in the formation of ion associates is calculated and the drug concentration is determined indirectly.

ANALYTICAL DETERMINATION OF MITFORMIN HCl IN PHARMA-CEUTICAL PREPARATIONS AND URINE SAMPLES

The metformin-containing pharmaceutical preparations (Glucophage, Glucophage XR and Diolan tablets) were successfully assayed using the present method. Sampling were made by grinding (5, 10 and 12 tablets) then taking 1.50-32.45, 2.50-34.25 and 2.75-33.50 µg / ml of the Glucophage, Glucophage XR and Diolan tablets, respectively at the optimum condition solution and the tablets were analyzed applying the above mentioned procedure. Urine samples were obtained from type II diabetic patients in Taif Area, Saudia Arabia (Ages from 40-55 years old) after 2, 6 and 10 hours of taking dose. In all cases the tablets and urine samples were analyzed applying the above described procedure.

RESULTS AND DISCUSSIONS

The results of elemental analysis (Table 2) of the produced solid ion associates reveal that two metformininium cations form ion associates with one $[M(SCN)_4]^{2-}$ while only one Mf combines with $[Cr(NH_3)_2(SCN)_4]^-$ to form a 1:1 ion associate. These results are comparable to the previously reported results.[37-40] Conductimetric titrations of the investigated inorganic complexes with Mf HCl were performed to give insight into the stoichiometric compositions of the ion associates formed in solutions. For all ion associates, the characteristic curves break at a molecular ratio ([Mf] / $[x]^{n-}$) of about 2, confirming the formation of 2:1 (Mf : x^{2-}) ion associates except in the case of the reineckate anion where the curve exhibits a sharp break at the 1:1 molecular ratio. The results obtained coincide with the elemental analysis of the precipitated ion associate.

EFFECT OF pH ON THE SOLUBILITY OF ION ASSOCIATES

The choice of a suitable pH value at which the ion associate exhibits the lowest solubility (Table 3) is of prime importance in the use of such compounds in quantitative analysis. To determine this pH value, the solubility and the solubility products of the compounds are determined at 25°C in solutions of varying pH values. From the obtained results, it was observed that increasing the pH value of the medium decreases the solubility of the ion associate, although only slightly, until a certain pH value (Table 3), when it then increases again. This can be explained by considering the solubility equilibrium of the ion associate, e.g.,

$$(Mf)_2[M(SCN)_4] \rightleftharpoons 2\,Mf^+ + [M(SCN)4]^{2-}.$$

In acid medium, the hydrogen ion may react with the complex anion, $[M(SCN)_4]^{2-}$, while in basic medium the hydroxyl ions may react with the metforminium ion or the metal thiocyanate complexes. However, it is of note that the effect of pH is rather weak and the present method can be applied safely over a wide range of pH values.

EFFECT OF IONIC STRENGTH ON THE SOLUBILITY OF ION ASSOCIATES

The choice of a suitable µ value at which the ion associates exhibit the lowest solubility is also of prime importance in the use of such ion associates in quantitative analysis. The solubility and the solubility product values of ion associates at different µ values (0.1-1.0) have been investigated at the optimum pH

values. It was found that increasing the μ value of the medium decreases the solubility of the ion associates, probably due to the salting out effect, until the optimum μ value is reached (Table 3). It then increases again due to complexation reactions between the base cations and the concentrated NaCl in the medium that form the drug precipitate, and hence the concentration of the metal ion increases, leading to an increase in the calculated solubility values. The values of the solubility and solubility product at the optimum conditions of pH and ionic strength (μ) are given in Table (3). The results indicate that the present ion associates are so sparingly soluble that Mf cit can be determined accurately and precisely by the indirect method through precipitation of its ion associates with [Cd(II), Mn(II) and Zn(II)] thiocyanate complexes, and ammonium reineckate.

ANALYTICAL DETERMINATION OF MITFORMIN HCl IN AQUEOUS SOLUTIONS, PHARMACEUTICAL PREPARATIONS AND URINE SAMPLES

Mf HCl was determined precisely and accurately in aqueous solutions and in the pharmaceutical preparation (Glucophage, Glucophage XR and Diolan tablets) using the present method. The results given in (Table 4) reveal that for ammonium reineckate the recovery is 100.26 %, reflecting a high accuracy which in addition to the high precision indicated by very low values of relative standard deviations. For (Cd, Mn(II) and Zn) thiocyanates, the recovery range is between 97.45 and 99.76 % -less accurate than that for ammonium reineckate. Generally, the present method is applicable over a wider concentration range; (0.33 - 36.42 μg / ml) than that of Narendra et al [34] and Nief et al [35-36] where 2 - 10, 10 - 100 and 2 - 20 μg/ml solution of Mf HCl can be determined, respectively. In pharmaceutical analysis it is important to test the selectivity toward the excipiences and the fillers added to the pharmaceutical preparations. Fortunately, such materials mostly do not interfere. This is clear from the results obtained for the pharmaceutical preparations (Table 4) that these excipiences do not interfere. Although the present method is more time consuming (22 min) in comparison to other methods such as (15 min for HPLC), it exhibits the advantages of simplicity, precision, higher sensitivity, accuracy and convenience. Moreover, the reproducibility of the results are superior to those obtained from other methods such as chromatography. [12, 17 and 20] Therefore, the method should be useful for routine analytical and quality control assay of the investigated drug in dosage forms. In order to establish whether the proposed method exhibits any fixed or proportional bias, a simple linear regression[43] of observed drug concentration against the theoritical values (five points) was calculated. Student' s *t*-test[44] (at 95% confidence level) was applied to slope of the regression line and showed that it didn't differ significantly from the ideal value of unity. Hence, it can be concluded that there are no systematic differences between the determination and true concentration over a wide range. The standard deviations (S.D.) can be considered satisfactory, at least for the level of concentrations examined.

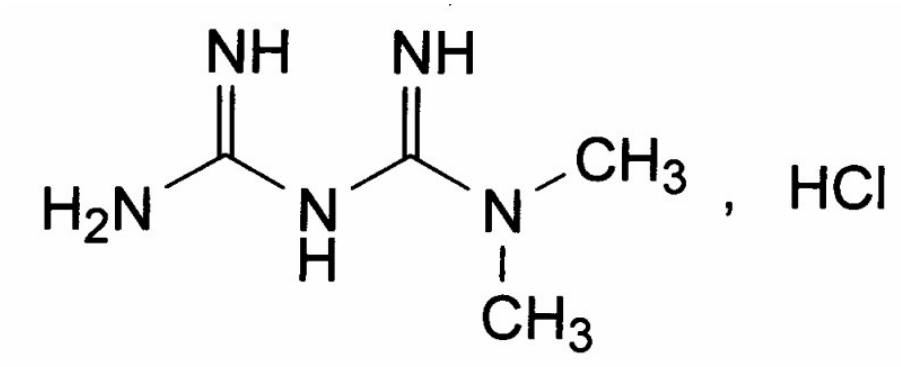

Figure 1
Chemical structure of Metformin. HCl

TABLE 1

Analytical Parameters for the Measurement of Cd, Cr, Mn and Zn Using ICP-AES

Element	Wavelength (nm)	Order	Plasma position	DL (mg/L)	LDR (mg/L)	BEC (mg)	RMF x BEC (%)
Cd	214.43	105	0	0.005	0.05-300	0.4	1 x 1.0
Mn	257.61	87	0	0.003	0.03-100	0.1	1 x 0.7
Cr	267.71	84	0	0.01	0.1-1000	0.4	7 x 0.7
Zn	206.20	109	0	0.01	0.1-1000	0.3	10x 0.9

Note. DL, detection limit; LDR, linear dynamic range; BEC, background equivalent concentration; RMF, relative standard deviation. For all elements: state, ion; entrance slits, 50 x 300 µm; exit slits, 100 x 300 µm.

TABLE 2

Elemental Analysis, Composition, and Some Physical Properties of Metformin hydrochloride Ion Associates

Ion-associate composition	m. p. °C	Molar ratio	Color	% found (calculated)				
				C	H	N	S	Metal Mn, Cd, Zn or Cr
$(C_4H_{11}N_5)_2[Mn(SCN)_4]$	246	2:1	white	26.46 (26.42)	4.07 (4.04)	36.02 (35.96)	23.50 (23.48)	10.12 (10.09)
$(C_4H_{11}N_5)_2[Cd(SCN)_4]$	238	2:1	white	23.87 (23.90)	3.61 (3.65)	32.49 (32.53)	21.21 (21.25)	18.61 (19.65)
$(C_4H_{11}N_5)_2[Zn(SCN)_4]$	274	2:1	white	25.88 (25.93)	3.91 (3.96)	35.25 (35.29)	23.01 (23.05)	11.71 (11.76)
$(C_4H_{11}N_5)[Cr(NH_3)_2(SCN)_4]$	290	1:1	pink	51.12 (51.17)	5.49 (5.54)	11.89 (11.94)	22.45 (22.54)	4.41 (4.05)

TABLE 3

Solubility and Solubility Product Values at 25°C of Mf HCl Ion Associates at Their Optimum pH and Ionic Strength (µ) Values

Ion Associate	pH	µ	pS	pK_{sp}
(Mf)2[Cd(SCN)$_4$]	4.0	0.3	2.61	7.23
$(Mf)_2[Zn(SCN)_4]$	3.0	0.5	3.24	9.14
$(Mf)_2[Mn(SCN)_4]$	5.0	0.6	3.03	8.63
$(Mf)[Cr(NH_3)_2(SCN)_4]$	2.0	0.4	4.25	8.50

Note. pS, -log solubility. pk_{sp}, -log solubility product.

TABLE 4
Analytical Determination of Metformin Hydrochloride in Aqueous Solution , Pharmaceutical Preparations and Urine Samples

Sample	Amount taken (µg)	Mean recovery (%)	Mean RSD (%)
$[Cd(SCN)_4]^{2-}$			
Pure Mf HCl solution	0.33 - 36.42	97.76	0.87
Glucophage Tablets[a]	1.50 - 32. 45	97.66	0.80
Glucophage XR tablets[b]	2.50 - 34.25	97.72	0.83
Diolan tablets[c]	2.75 - 33.50	97.55	0.81
Urine after 2 hrs	22.50 – 35.25	97.88	0.77
Urine after 6 hrs	15.45 – 28.35	97.66	0.86
Urine after 10 hrs	4.35 – 10.55	97.45	0.82
$[Zn(SCN)_4]^{2-}$			
Pure Mf HCl solution	0.33 - 36.42	98.76	0.87
Glucophage Tablets[a]	1.50 - 32. 45	98.46	0.83
Glucophage XR tablets[b]	2.50 - 34.25	98.71	0.80
Diolan tablets[c]	2.75 - 33.50	98.76	0.87
Urine after 2 hrs	22.50 – 35.25	98.66	0.55
Urine after 6 hrs	15.45 – 28.35	98.45	0.45
Urine after 10 hrs	4.35 – 10.55	98.56	0.57
$[Mn(SCN)_4]^{2-}$			
Pure Mf HCl solution	0.33 - 36.42	99.71	0.97
Glucophage Tablets[a]	1.50 - 32. 45	99.76	0.88
Glucophage XR tablets[b]	2.50 - 34.25	99.73	0.87
Diolan tablets[c]	2.75 - 33.50	99.68	0.84
Urine after 2 hrs	22.50 – 35.25	99.56	0.89
Urine after 6 hrs	15.45 – 28.35	99.63	0.85
Urine after 10 hrs	4.35 – 10.55	99.65	0.82
$[Cr(NH_3)_2(SCN)_4]^{-}$			
Pure Mf HCl solution	0.33 - 36.42	100.26	0.54
Glucophage Tablets[a]	1.50 - 32. 45	100.25	0.65
Glucophage XR tablets[b]	2.50 - 34.25	100.15	0.67
Diolan tablets[c]	2.75 - 33.50	100.22	0.62
Urine after 2 hrs	22.50 – 35.25	100.23	0.66
Urine after 6 hrs	15.45 – 28.35	100.28	0.65
Urine after 10 hrs	4.35 – 10.55	100.21	0.67

Note. RSD, relative standard deviation (six determinations).
(a) Merck Sante S. A. S., France
(b) Merck Serono, KGaA, Germany
(c) Gulf Phrmaceutical Industries, Ras Al Khaimah, U.A.E.

CONCLUSION

The present method is applicable over a wider concentration range; (0.33 - 36.42 µg / ml) than that of Narendra et al [34] and Nief et al [35-36] where 2 - 10, 10 - 100 and 2 - 20 µg/ml solution of Mf HCl can be determined, respectively In pharmaceutical analysis it is important to test the selectivity toward the excipiences and the fillers added to the pharmaceutical preparations. Fortunately, such materials mostly do not interfere. This is clear from the results obtained for the pharmaceutical preparations (Table 4) that these excipiences do not interfere. As with any medication, it is possible to overdose on metformin. Some of the effects of a metformin overdose may include low blood sugar or lactic acidosis. Symptoms of low blood sugar include blurred vision, shakiness, and extreme hunger. Some symptoms of lactic acidosis can include an irregular heartbeat, trouble breathing, and feeling tired. There are some treatment options for a metformin overdose, including dialysis or using a sugar solution to increase blood sugar levels.

Although the present method is more time consuming (22 min) in comparison to other methods such as (15 min for HPLC), it exhibits the advantages of simplicity, precision, higher

sensitivity, accuracy and convenience. Moreover, the reproducibility of the results are superior to those obtained from other methods such as chromatography. [12, 17 and 20] Therefore, the method should be useful for routine analytical and quality control assay of the investigated drug in dosage forms.

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