Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2013, 5(6):156-163
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Preparation, characterization and atomic absorption spectroscopic determination of some metal complexes of glipizide

Hassan R. H. Ali[a*], Gamal A. Saleh[a], Samiha A. Hussein[a] and Ahmed I. Hassan[b]

[a]*Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Assiut University, Assiut, Egypt*
[b]*Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Al-azhar University (Assiut Branch), Assiut, Egypt*

ABSTRACT

Glipizide is a commonly used sulfonylurea oral hypoglycemic drug. The present work reports the preparation and characterization of its metal complexes with cadmium, mercuric, ferric, zinc, cobalt, nickel and calcium. The existence of these complexes has been confirmed by FTIR spectroscopy, which revealed that formation of these complexes was in 2:1 stoichiometry of glipizide and each studied metal, respectively. Close FTIR inspection suggested the co-ordination of nitrogen and carbonyl oxygen atom of –SO_2NHCONH- moiety of glipizide with the studied metals. Further, the formed complexes have been analyzed using atomic absorption spectroscopy. The method was successfully applied for the indirect determination of glipizide in its dosage form for the first time.

Keywords: Glipizide, metal complex, FTIR, sulfonylurea, atomic absorption

---

INTRODUCTION

Glipizide (N-[2-[4-[[[(cyclohexylamino) carbonyl]amino]sulfonyl]phenyl] ethyl]-5-methyl pyrazinecarboxamide); GPZ, Figure 1, is one of oral medium-to-long acting sulfonylurea hypoglycemic drug. It is acting by partially blocking potassium channels in the beta cells of the islets of Langerhans. Thus, it will increase the time that the cell spends in the calcium release stage of cell signaling leading to an increase in calcium, which will initiate more insulin release from each beta cell [1].

A perusal of available literature shows that; systemic study on complexation of metals with sulphonylurea was carried out by Iqbal and coworkers [2]. It has been reported that the biological activity of the metal complex is more potent and less toxic as compared to the free drug or in other words, many drugs possessed modified pharmacological and toxicological properties upon metallic complexation [3,4]. Metals like, zinc, copper, selenium, could lower the blood sugar level [5]. The metal complexes of some sulphonylurea oral hypoglycemics, gliclazide [6], glibenclamide [7], and glimepiride [8] have been reported.

Alan Walsh had developed atomic absorption spectroscopy (AAS) in the early 1950' [9]. It works on the principle that certain elements absorb certain wavelengths, and this level of absorption is characteristic of each element. AAS is a very accurate and reliable technique, because even if there is contamination of other elements in the sample, it will still only measure the concentration of the specific element being evaluated. This technique determines the exact concentration, as low as 0.1ppm. AAS is one of the chemist's favorite analytical tools for quantitative trace analysis of metallic elements in various samples alloys, rocks, soils, foods, drinks, biological fluids, and chemicals. AAS has been widely utilized for the indirect determination of many pharmaceuticals [10-15].

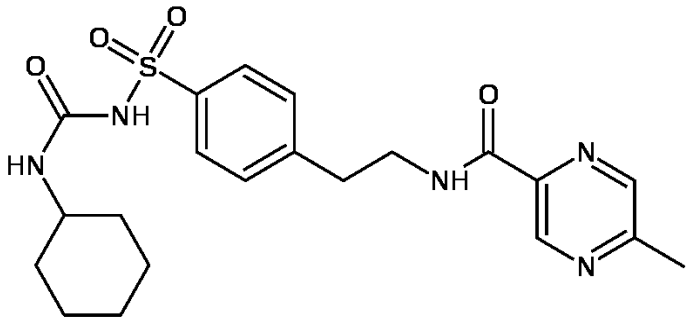


Figure 1: Chemical structure of GPZ

Despite the possibility of administration of various metals as food supplement for diabetic patients under GPZ which could affect their blood sugar levels, there is no reported investigation of the complexation of GPZ with some commonly administered metals. In the present work, novel metal complexes of GPZ with copper, ferric, zinc, cobalt, nickel, manganese, calcium and magnesium in addition to cadmium and mercuric were prepared and their existence was confirmed by FTIR spectroscopy. Further, the formed complexes have been analyzed by AAS for the indirect determination of GPZ in its dosage forms for the first time.

MATERIALS AND METHODS

Chemicals

Glipizide was purchased from Sigma Aldrich Gillingham Dorset, England. Potassium bromide was purchased from El-Nasr Pharmaceutical Chemical Co., Abo-Zaabal, Egypt. All metals, solvents and other chemicals were of analytical grade and used as received.

Pharmaceuticals

Glipizide® tablets (Pharco Pharmaceuticals, Alexandria, Egypt) are labeled to contain 5 mg of GPZ per tablet.

Preparation of metal complexes

Accurately weighed amounts of GPZ 0.445 g (1.0 mM) and potassium hydroxide 0.056 g (1.0 mM) were dissolved in 50 ml of ethanol by stirring for 15 min at room temperature. Ethanolic solutions of 0.05 and 0.10 mM from each metal chloride separately were added dropwise to GPZ solutions while stirring. Initially clear solutions were formed. The reaction mixtures were refluxed for an hour and then their volumes were reduced to 30 ml by slow evaporation on a water bath at 40 °C. After settling down at room temperature, the products obtained were filtered, washed with ethanol and acetone and dried at 50°C overnight. The solid phase identity of the products was confirmed by melting point determination and FTIR spectroscopy.

Treatment of the pharmaceutical dosage forms

The extraction of GPZ from pharmaceuticals in tablet form was achieved through the selective dissolution of GPZ with ethanol according to the British Pharmacopeia [16]. Twenty tablets were weighed, and finely powdered. An accurately weighed quantity of the powdered tablet was transferred into a 100 ml calibrated flask, and dissolved in about 50 ml of ethanol. The contents of the flask were swirled, sonicated for 5 min and then completed to the volume with ethanol. The mixtures were mixed well, filtered and the first portion of the filtrate was rejected. A measured volume of the prepared solution was diluted quantitatively with ethanol, and the resulting solution was used for analysis by the recommended procedures.

Determination of GPZ- metal complexes by AAS

Solutions for the determination of metal contents were prepared by digesting 0.040 g of each metal complex by 5 ml of concentrated hydrochloric acid then the volume were diluted to 100 ml with double distilled water in 100 ml calibrated flasks. The working solutions were obtained by further dilution of this stock solution with distilled water and amount of metals was measured against the blank solution using AAS.

Instrumentation

Melting Point Determination

The melting points of GPZ and its metal complexes were determined using a Digital Electro-thermal Melting Point Apparatus, England, with 2 ºC interval scale and sealed capillary method.

Infrared spectroscopy
FTIR spectra were collected using a Nicolet 6700 FTIR Advanced Gold Spectrometer with OMNIC 8 software. The FTIR spectra of the investigated drug and its metal complex were recorded as potassium bromide discs (1:200) in the range of 4000-400 cm^{-1} at 4 cm^{-1} spectral resolution with the accumulation of 256 spectral.

Spectral preprocessing
All the FTIR spectra were exported to the Galactic SPC format and manipulated using GRAMS AI (Galactic Industries, Salem, NH, USA, version 7.01).

Atomic absorption spectroscopic studies
Atomic absorption spectroscopic studies of the formed GPZ-metal complexes were carried out using MODEL 210 VCP atomic absorption spectrophotometer (Buck Scientific, USA), detection limit 0.1 ppm and integration time 3 s. The flame used was acetylene-air mixture. A Shimadzu atomic absorption flame spectrophotometer model AA.640-13, slit width 0.2 nm, relative noise 1.0, detection limit 0.6 ppm and integration time 3 s. The flame used was an acetylene-air mixture.

RESULTS AND DISCUSSION

The physical characteristics of the products of the interaction between GPZ and copper, cadmium, mercuric, ferric, zinc, cobalt, nickel, manganese, calcium and magnesium were presented in Table 1.

Table 1: The physical characteristics of the products of the interaction between GPZ and metals

S. No.	Compound	Color	State	Melting point (ºC)	Yield (%)
1	GPZ free	White	Crystalline	203-205	
2	GPZ- Cu(II) complex	Dark brown	Crystalline	> 300	67.4
3	GPZ- Cd(II) complex	Off white	Crystalline	> 300	73.9
4	GPZ- Hg(II) complex	White	Amorphous	> 300	86.7
5	GPZ- Fe (III)complex	Reddish brown	Crystalline	> 300	83.9
6	GPZ- Zn(II) complex	Grayish White	Amorphous	> 300	74.8
7	GPZ- Co(II) complex	Blue	Crystalline	> 300	77.4
8	GPZ- Ni(II) complex	Green	Crystalline	> 300	85.9
9	GPZ- Mn(II) complex	Brown	Crystalline	> 300	70.8
10	GPZ- Ca(II) complex	White	crystalline	> 300	88.3
11	GPZ- Mg(II) complex	Off white	Amorphous	> 300	63.5

FTIR spectroscopic analysis
The existence of the metal complexes of GPZ has been investigated by FTIR spectroscopy. Our earlier study has presented a comprehensive FTIR spectroscopic investigation of GPZ [17]. The key FTIR spectral features of GPZ are υ $(NH)_{amide}$ band at 3326 cm^{-1}, υ $(NH)_{urea}$ band at 3249 cm^{-1}, υ (C=O) band at 1690 cm^{-1}, δ (CCC) band at 1527 cm^{-1}, δ (CCH) band at 1485 cm^{-1}, υ (SO_2) band at 1159 cm^{-1} and δ (CO) band at 607 cm^{-1}. By close inspection of the FTIR spectra of GPZ and its respective products with Cu (II), Cd (II), Hg (II), Fe (III), Zn (II), Co (II), Ni (II), Mn (II), Ca (II) and Mg (II) in 1:1 molar ratio has shown that there is no significant changes in their FTIR spectra which could indicate that there is no complexation in this ratio. On the other hand in 2:1 molar ration there were significant differences between FTIR spectra of GPZ and its respective products will all the aforementioned metals except for Cu (II), Mn (II) and Mg (II), which could indicate the existence of complexation between GPZ and these metals. The FTIR spectra of GPZ and its respective products with Cd (II) and Ni (II) in 1:1 and 2:1 molar ratio were presented in Figure 2 as representative examples. These differences in the FTIR spectra will be discussed in details in the following subsection.

The FTIR spectra of GPZ and its respective products are shown in Figures 2 and 3. A number of differences in peak shape, intensity and position are immediately apparent. It can be seen that, the strong υ $(NH)_{urea}$ and the very strong υ $(C=O)_{urea}$ bands at 3249 and 1690 cm^{-1}, respectively, in the FTIR spectrum of GPZ has disappeared in the FTIR spectra of it respective products with all the studied metals except Cu (II), Mn (II) and Mg (II Figures 2 and 3, [18]. The disappearance of these bands could suggest that the complexation has occurred through nitrogen and carbonyl oxygen atom of $–SO_2NHCONH–$ moiety of GPZ in 2:1 molar ratio, Scheme 1.

Interestingly, the $\upsilon(NH)_{amide}$, $\upsilon(C=O)_{amide}$ and $\upsilon(SO_2)_{sym}$ bands were recorded in the FTIR spectra of the metal complexes of GPZ with different positions and intensities, Table 2, which could confirm the suggested pathway of the complexation process, Scheme 1. New bands were observed in the region 650-790 cm^{-1} in the FTIR spectra of GPZ complexes which could be attributed to $\upsilon(M-O)_{complex}$ and $\upsilon(M-N)_{complex}$ [18,19]. The recording of these bands is also diagnostic for the complex formation, Table 2.

It is appropriate here to mention that there were no significant differences between FTIR spectra of GPZ and its respective products with Cu (II), Mn (II) and Mg (II). The failure of complexation of GPZ with these metals may be attributed to the weakness of the formed products [20] and hence, no further analytical studies were carried out for these particular metals.

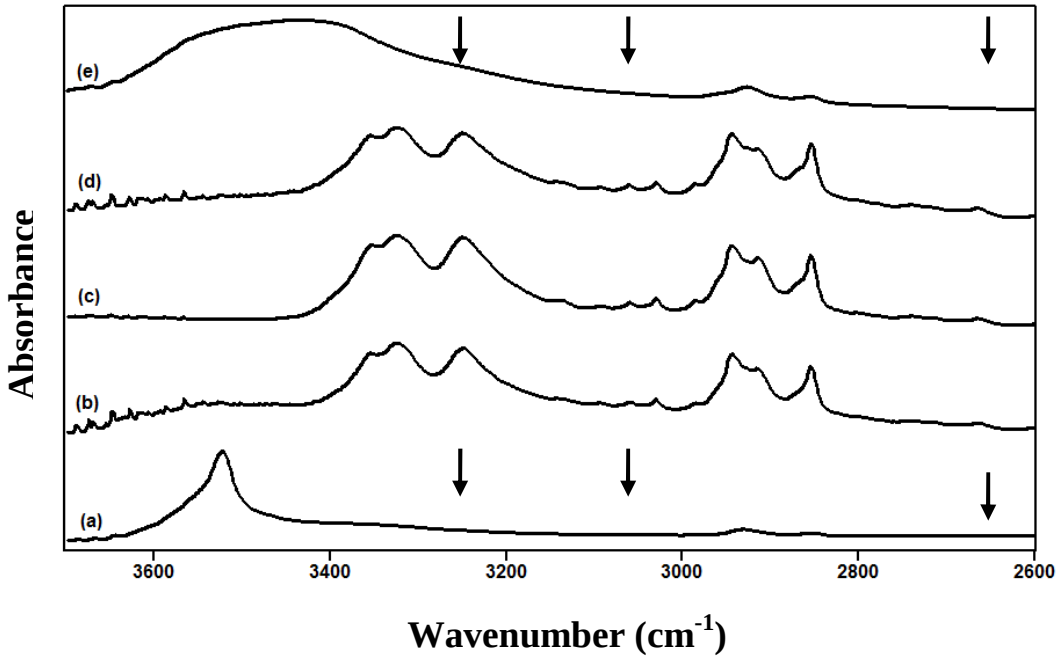


Figure 2a: The FTIR spectra of (a) GPZ-Cd(II) in 2:1 ratio, (b) GPZ-Cd(II) in 1:1 ratio, (c) pure GPZ , (d) GPZ-Ni(II) in 1:1 ratio, and (e) GPZ-Ni(II) in 2:1 ratio in the region of 2600-3700 cm^{-1}

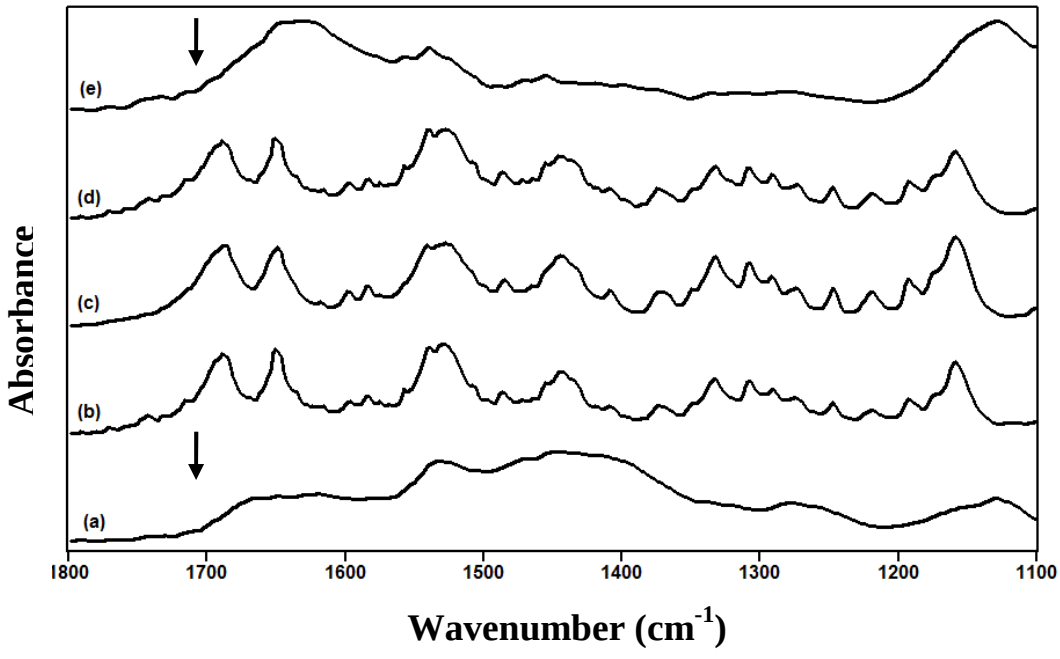


Figure 2b: The FTIR spectra of (a) GPZ-Cd(II) in 2:1 ratio, (b) GPZ-Cd(II) in 1:1 ratio, (c) pure GPZ , (d) GPZ-Ni(II) in 1:1 ratio, and (e) GPZ-Ni(II) in 2:1 ratio in the region of 1100-1800 cm^{-1}

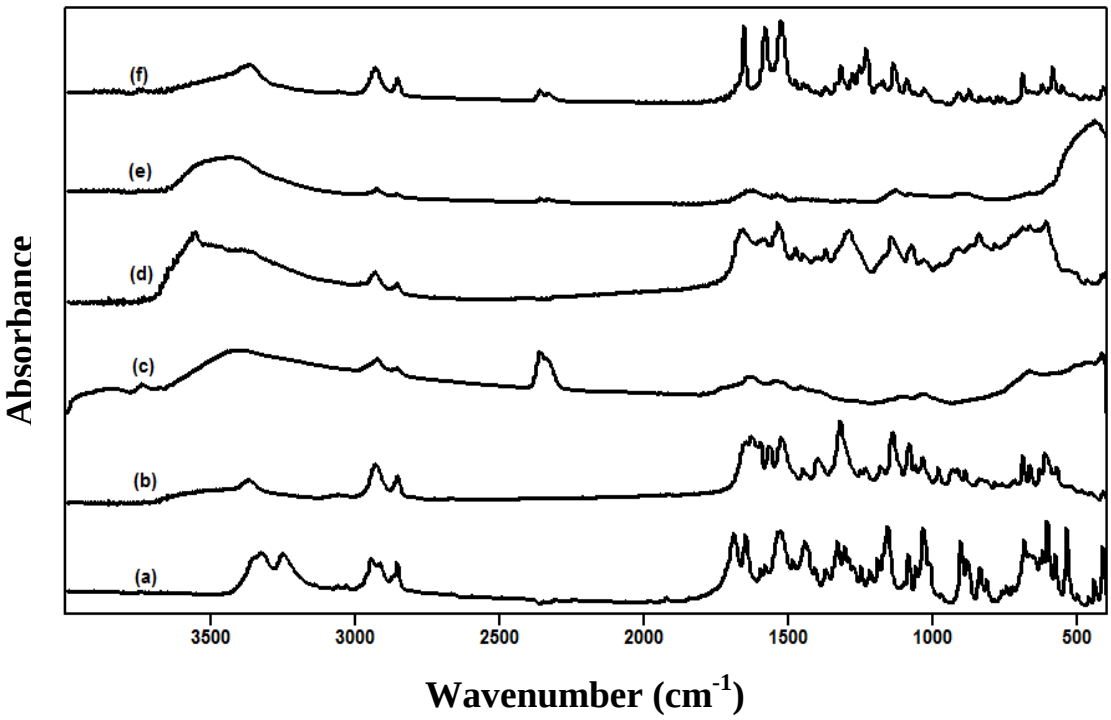


Figure 3: The FTIR spectra of (a) pure GPZ, (b) GPZ-Hg(II), (c) GPZ-Fe(III), (d) GPZ-Co(II), (e) GPZ-Zn(II) and (f) GPZ-Ca(II) in 2:1 ratio in the region of 400-4000 cm^{-1}

Table 2: The distinctive FTIR wavenumbers of GPZ and its metal complexes

FTIR wavenumber (cm^{-1})											Proposed assignment
GPZ Free	**GPZ- Cu(II)**	**GPZ- Cd(II)**	**GPZ- Hg(II)**	**GPZ- Fe(III)**	**GPZ- Zn(II)**	**GPZ- Co(II)**	**GPZ- Ni(II)**	**GPZ- Mn(II)**	**GPZ- Ca(II)**	**GPZ- Mg(II)**	
		3520 *vs*		3410 *vs*	3441 *m*	3555 *vs*	3450 *vs*				υ(OH)
3326 *s*	3324 *s*	3395 *s*	3370 *m*	3398 *vs*	3393 *m*	3395 *s*	3390 *s*	3326 *s*	3363 *ms*	3353 *s*	$\upsilon(NH)_{amide}$
3249 *s*	3251 *s*							3249 *s*		3252 *s*	$\upsilon(NH)_{urea}$
1690 *vs*	1689 *vs*							1689 *vs*		1689 *vs*	$\upsilon(C=O)_{urea}$
1650 *vs*	1650 *vs*	1649 *m*	1647 *vs*	1670 *s*	1634 *m*	1661 *vs*	1644 *vs*	1650 *vs*	1655 *vs*	1652 *vs*	$\upsilon(C=O)_{amide}$
1158 *vs*	1145 *vs*	1133 *m*	1140 *vs*	1155 *ms*	1128 *m*	1143 *vs*	1125 *ms*	1159 *vs*	1138 *ms*	1161 *vs*	$\upsilon(SO_2)_{sym}$
		784 *ms*	786 *m*	780 *m*	776 *mw*	785 *s*	785 *ms*		779 *mw*		$\upsilon(M-O)_{complex}$
		661 *ms*	665 *ms*	668 *vs*	669 *mw*	666 vs	666 *m*		666 *mw*		$\upsilon(M-N)_{complex}$

m, s, v and w stand for medium, strong, very and weak respectively. υ and δ stand for stretching and bending, respectively.

Atomic absorption spectroscopy

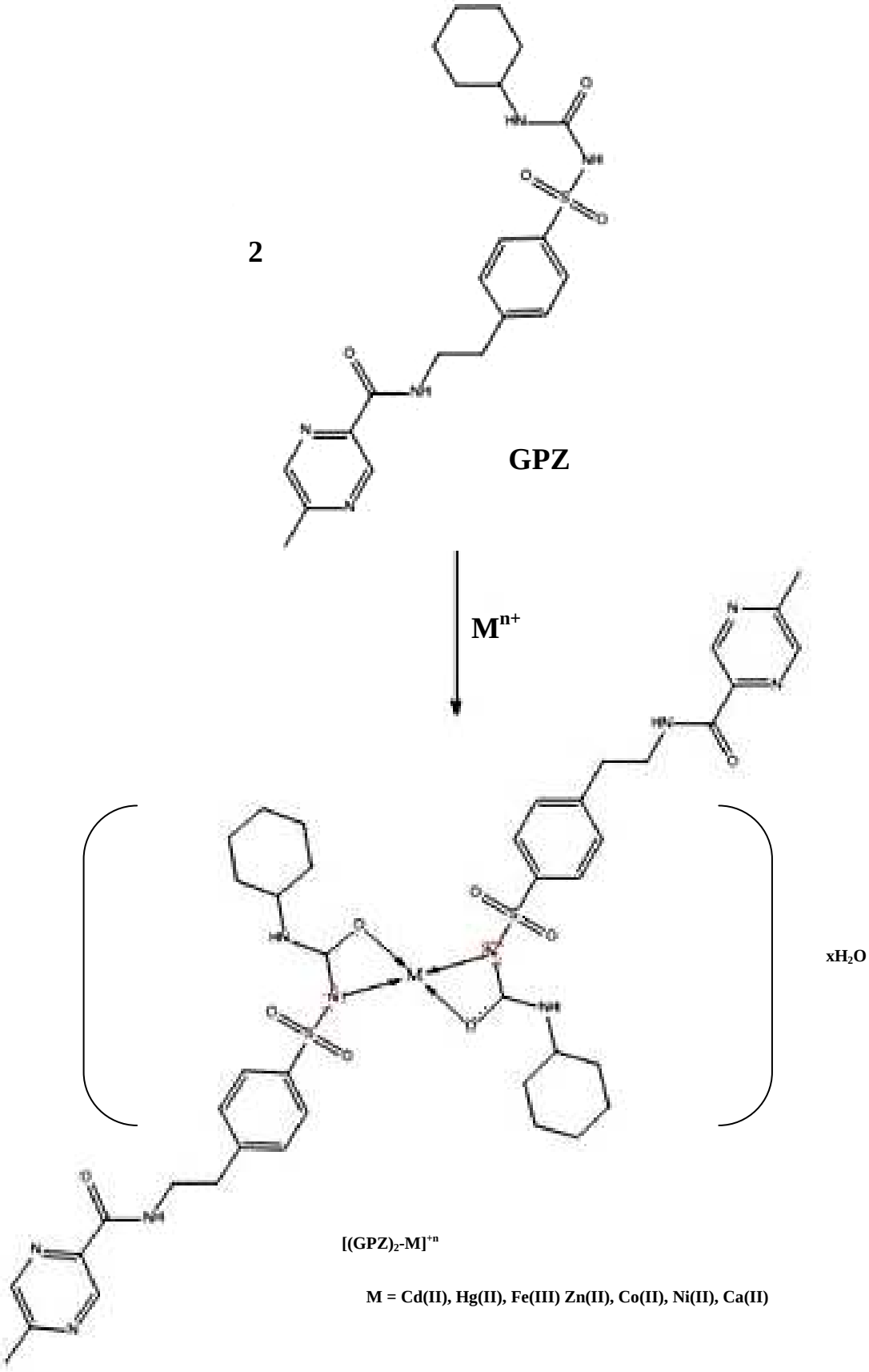
Estimation of metal content

Atomic absorption analysis was carried out by direct method to estimate the total metal content. A number of reference standard solutions of each metal were prepared in various concentration ranges. Absorbances of these solutions were measured at the specific wavelength of each metal using background correction technique. Calibration graphs were plotted for metals solutions. The concentrations of unknown solutions were calculated from their respective absorbances by using the standard values. Results of analysis are presented in Table 3.

Table 3: Estimation of metals in GPZ-metal complexes by AAS

Complex	Metal wavelength (nm)	Metal calculated (ppm)	Metal found ± S. D[a] (ppm)
GPZ- Fe(III)	248	25.1	24.5 ± 0.012
GPZ- Zn(II)	214	10.0	9.72 ± 0.019
GPZ- Co(II)	240	25.0	25.2 ± 0.053
GPZ- Ni(II)	232	25.0	24.8 ± 0.032

[a] *SD = standard deviation*



Scheme 1: The suggested pathway of the interaction between GPZ and metals

Quantitative determination and validation

In the present work, GPZ is found to react with Zinc(II) chloride to form suitable molecular complex, Table 3. This complex was selected, due to its apparent sensitivity, for the quantitative AAS indirect determination of GPZ by measuring zinc absorption at 214 nm. The developed procedures were validated according to USP *XXV* validation guidelines [21] and International Conference on Harmonization (ICH) guidelines [22]. The following validation parameters were studied:

Linearity, detection and quantitation limits

The calibration curve of zinc in its formed complex with GPZ was constructed by analyzing a series of concentrations of the standard solutions, and then plotting the absorbance as a function of their corresponding concentrations. The data were analyzed by least square method. Linear relationship was found between the measured values of absorbance and the concentrations of the metal. The data that obtained from the calibration curve of the metal was used for the indirect calibration of GPZ. High correlation coefficient values were obtained (0.9998) in the general concentration range of 11-40 µg. LODs and LOQs were found to be 3.60 and 11.0 µg, respectively. Table 4 summarizes the results obtained from analyzing the calibration curve.

Table 4: Quantitative parameters for the assay of GPZ in GPZ-Zn(II) complex by AAS

Parameter [a]	GPZ
Linear range	11-40 (µg)
Intercept (a) ± SD	-0.00536 ± 0.00386
Slope (b) ± SD	0.02093 ± 0.00017
Correlation coefficient (r)	0.9998
LOD	3.60 (µg)
LOQ	11.0 (µg)

[a] *n= three determination LOD : limit of detection LOQ: limit of quantitation*

Accuracy and precision

The value of t-test in determination of GPZ has indicateded that, there is no significant difference in determination of GPZ by the proposed method, which indicates its high accuracy. The precision of the proposed method was determined by carrying out replicate analysis of three samples of the drug. The relative standard deviations did not exceed 2% and thus indicating good reproducibility of the proposed methods, Table 5. This precision level is adequate for the routine analysis of the investigated drugs in quality control laboratories and routine pharmaceutical analysis.

Table 5: Precision of the proposed AAS method

Drug	Conc. (µg)	Absorbance[a]			Mean	SD[b]	RSD[c] (%)
		Sample number					
		1	2	3			
GPZ	35	0.677	0.655	0.680	0.670	0.0111	1.65

[a] *Results are compared with those of standard calibration curves.*
[b] *SD: standard deviation.*
[c] *RSD: relative standard deviation.*

Ruggedness

Ruggedness was assessed by applying the proposed method to the assay of the investigated drug using the same procedures but with two different AAS instruments at two different laboratories and different elapsed time. The results obtained from lab- to-lab and day-to-day variations were found to be reproducible, as RSD did not exceed 2%, Table 6.

Table 6: Ruggedness of the proposed AAS method

Drug	Recovery (% ± SD)[a]			
	Instrument		**Inter-day variation**	
	VCP AA	Shimadzu AA	1 day	2 day
GPZ	99.6 ± 0.89	99.2 ± 1.03	100.5 ± 0.95	99.6 ± 0.89

[a] *Values are the mean of three determinations ± SD.*

Application to the analysis of the pharmaceutical tablets

The aforementioned results shows that the proposed method produces satisfactory results with GPZ in pure form. Thus, GPZ tablets were subjected to the analysis of their contents of the drug by the proposed AAS method. The recovery percentage was found to be 99.3 ± 1.16%, Table 7. The results were compared statistically with those obtained by the reported methods [23] with respect to the accuracy (t-test) and precision (F-test). No significant

differences were found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level. This indicated similar accuracy and precision in the analysis of GPZ in its tablets.

Table 7: Analysis of GPZ in its tablets using the proposed AAS and the reported methods

Product	Recovery (%± SD)		F-value[a]	t-value[a]
	Proposed method	**Reported method**		
Glipizide® tablets	99.3±1.16	98.1±1.25	1.19	1.54

[a] *Theoretical values for t and F at 95% confidence limit (n = 5) were 2.78 and 6.39, respectively.*

CONCLUSION

The preparation and characterization of the metal complexes of GPZ with Cd (II), Hg (II), Fe (III), Zn (II), Co (II), Ni (II) and Ca (II) and were presented for the first time. The existence of these complexes has been confirmed by FTIR spectroscopy, which revealed that formation of these complexes was in 2:1 stoichiometry of GPZ and each studied metal. The FTIR spectroscopic analysis has suggested the co-ordination of nitrogen and carbonyl oxygen atom of -SO_2NHCONH- moiety of GPZ with the studied metals. Further, the formed complexes have been analyzed by AAS. The method was successfully applied for the indirect determination of GPZ in its tablets for the first time.

Acknowledgment
The authors wish to thank the members of the center of pharmaceutical services, faculty of pharmacy, Assiut University for their technical support.

REFERENCES

[1] C. Dollery, Therapeutic Drugs, 2nd edition, Churchill Livingstone, Edinburgh, London, **1999**, 1, G65.
[2] S. A. Iqbal, S. Jose, I. Zaafarany, *Orient. J. Chem*., **2012**, 28, 613-618.
[3] Z. Guo, P. J. Sadler, *Angew. Chem. Int. Ed*., **1999**, 38, 1512-1531.
[4] W. N. Lipscomb, N. Sträter, *Chem. Rev*., **1996**, 96 , 2375-2433.
[5] A. B. Chausmer, Zinc, *J. Am. Coll. Nutr*., **1998**, 17 , 109-115.
[6] M. S. Arayne, N. Sultana, M. K. Zaman, A. Farooq, *Pak. J. Pharm. Sci*., **2005**, 1, 35-40.
[7] Kh. Rasheed, M. Tareq, Ch. Munir, I. Hussain, H. L. Siddiqui, *Chem. Pharm. Bull*., **2008**, 56, 168-172.
[8] S. A. Iqbal, S. Jose, J. George, Synthesis, *Orient. J. Chem*, **2011**, 27 , 731-735.
[9] A. L. El-Ansary, W. F. El-Hawary Y. M. Issa, A. F. Ahmed, *Anal. Lett*., **1999**, 32, 2255-2269.
[10] L. M. Abdellaziz, M. M. Hosny, *Anal Chem Insights*., **2011**, 6, 67–78.
[11] N. M. El-Kousy, L. I. Bebawy, *J. Pharm. Biomed. Anal*., **1999**, 20, 671–679.
[12] F. M. Abdel-Gawad, *J. Pharm. Biomed. Anal*., **1998**, 16, 793–799.
[13] S. Khalil, A. Kelzieh, *J. Pharm. Biomed. Anal*., **2002,** 27, 123–131.
[14] S. M. Anise, M. M. Hosny, H. E. Abdelatef, M. N. EL-Balkiny, *Chem. Ind. Chem. Eng*. Q., **2011**, 17, 269−282.
[15] M. K. Hammood, A. W. Qasim, F. Jasim, *Nat. J. of Chem*., **2011**, 41, 27-37.
[16] British Pharmacopoeia, the Stationery Office, London, **2012**.
[17] H. R. Ali, G. A. Saleh, S. A. Hussein, A. I. Hassan, *Anal. Chem. Ind. J*. **2013**, in press.
[18] M. AL-Majthoub, M. Salman, *J. Chem. Pharm. Res*., **2012**, 4, 1856-1863.
[19] Sh. A. Shaker, Y. Farina, *Mod. Appl. Sci*., **2009**, 3 ,88-93.
[20] A. A. Athawale, M. Majumdar, H. Singh, K. Navinkiran, *Def. Sci. J*. **2010**, 60 ,507-513.
[21] United State pharmacopoeia 25, The National Formulary 20th Edition, US, Pharmacopoeial Convention, Rockville, MD, **2002**.
[22] Topic Q2A: Text on Validation of Analytical Procedures, International Conference of Harmonization, **2005**.
[23] R. R. Sarangi, S. N. Panda, S. K. Panda, K. C. Sahu, *Int. J. Pharm. Bio. Arch*., **2011**, 2, 1137- 1145.