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MICRO-DETERMINATION OF TADALAFIL IN PHARMACEUTICAL FORMULATIONS AND URINE SAMPLES OF DIABETIC PATIENTS TYPE-II IN TAIF AREA, SAUDIA ARABIA USING ATOMIC ABSORPTION AND ATOMIC EMISSION SPECTROMETRY

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ABSTRACT

New ion – associate complexes of tadalafil hydrochloride with [Cadmium (II) and Zinc (II)] thiocyanates were precipitated and the excess unreacted metal complex was determined. A new method was given for the determination of tadalafil drug in pure solutions, in pharmaceutical formulations and urine samples of diabetic patients type – II using atomic emission and atomic absorption spectrometry. The drug can be determined by the affort method in the range 0.32 - 160.65 µg mL^{-1}.

KEYWORDS: **Atomic emission, atomic absorption, ion-associate complexes, pharmaceutical analysis.**

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INTRODUCTION

Now, The oral pharmacotherapy used for the treatment of the numerous number of patients who suffer from erectile dysfunction is represented by phosphodiesterase type 5 (PDE5) inhibitors, of which three drugs are currently used all over the world. Sildenafil,the first drug was approved in 1998. Recently, tadalafil and vardenafil were introduced through 2003 and 2004, respectively. Vardenafil is a potent and selective inhibitor of PDE5.[1,2] Tadalafil; (Td) is a very important pharmaceutical compound. Therefore, we found it important to prepare new ion-associates containing this drug and to study and elucidate their chemical structures. Also the work present a new rapid method for the determination of this drug after transformation into the ion-associates. Tadalafil is highly selective phosphodiesterase type 5 inhibitor and may not affect other parts of the body e.g. brain, heart, kidney and eyes. It does not require any intercourse planning as it allows spontaneity due to its long duration of action, giving more natural relationship feeling. Td is chemically known as pyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione,6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahy-dro-2-methyl-,(6R-trans)-(6R-,12aR)-2,3,6,7,12,12a-hexahydro-2-methyl-6-[3,4-(methylenedioxy)phenyl]-pyrazino[1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione. The chemical structure of tadalafil drug is shown in Figure (1). To the best of our knowledge few reports have been published on the analysis of tadalafil in pharmaceutical preparations.[3 - 9] The use of simpler, faster, less expensive and sensitive method is required. Although, Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES) and Atomic Absorption Spectrometry (AAS) are rapid methods and have a very low detection limits which can not be reached by most of other methods. The present study includes new ICP-AES and AAS methods for the determination of the investigated drug. The method is based on the precipitating of the ion-associates formed as a result of the combination of this drug with an excess of $[Cd(SCN)_4]^{2-}$ and $[Zn(SCN)_4]^{2-}$. The equilibrium concentration of the metal ion present as the soluble inorganic complex ion in the supernatant solution was determined using atomic emission and absorption.

MATERIALS AND METHODS

Doubly-distilled water and analytical grade reagents were used in the preparation of all solutions. Tadalafil was obtained from Eli Lilly and Company, USA. Cialis® tablet (containing 20 mg of tadalafil), manufactured by Eli Lilly and Company, USA and Snafi tablets (containing 20 mg of tadalafil), manufactured by Al- Qassim Pharmaceutical Plant, Saudi Arabia (SPIMACO) were purchased from local market. potassium thiocyanate was from Aldrich(www.sigmaaldrich.com).

APPARATUS

The pH of the solutions was measured using an Orion Research Model 701A digital pH-meter. Inductively 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 THE STANDARD SOLUTIONS

Standard solutions of divalent cadmium and zinc were prepared by weighing 1.0 g of high purity sample (cadmium and zinc, respectively), transferring it to a 1-liter measuring flask and then adding 50 ml of concentrated HNO_3. After complete dissolution, the solution was filled to the mark with distilled water. The 1000 µg mL^{-1} solution was stored in plastic bottles which had been presoaked in dilute HNO_3. The solutions were stable for approximately one year.

EMISSION AND ABSORPTION MEASUREMENTS

Analytical Parameters for the Measurement of Cd and Zn Using ICP-AES are listed in Table 1. Using AAS the Zn (II) was measured at wavelength 213.9 nm, slit 0.7 nm, relative noise 1.0, sensitivity 0.018 µg $mL^{-1}$and linear range 1.0 µg mL^{-1}. The instruments were equally adequate for present purposes and were used according to availability. The atomic spectrometry was calibrated as in the previously reported work.[4,5,10]

DETERMINATION OF SOLUBILITY OF THE ION – ASSOCIATES

The solid ion-associate was added in excess to a solution of the optimum pH and ionic strength. The solution was shaken for 4-6 hrs and left to stand for a weak to attain equilibrium. Then the saturated solution was filtered into a dry-beaker (rejecting the first few ml of filtrate). The equilibrium concentration of the metal ion present in the form of a soluble inorganic complex was measured using atomic spectrometry. Hence the solubility (S) of the precipitate was evaluated, from which the solubility product of the ion-associate was calculated.

CONDUCTOMETRIC MEASUREMENTS

The stoichiometry of the ion-associates was elucidated also by conductometric titrations [11] of the drugs with $[Cd(SCN)_4]^{2-}$ and $[Zn(SCN)_4]^{2-}$ solutions.

ANALYTICAL DETERMINATION OF TADALAFIL IN AQUEOUS SOLUTIONS

Aliquots (0.015 - 7.5 mL) of 0.001 mol L^{-1} drug solutions were quantitatively transferred to 25 mL volumetric flasks. To each flask 1.0 mL of 0.01 mol L^{-1} standard solution of $[Cd(SCN)_4]^{2-}$ and $[Zn(SCN)_4]^{2-}$ was added and the volume was completed to the mark with the aqueous solutions of the optimum pH and ionic strength (prepared from HCl and NaOH). The solutions were shaken well and left to stand for 15 min then filtered through Whatman P/S paper (12.5 cm). The equilibrium metal ion concentration in the filtrate was determined using ICP-AES or AAS. The consumed metal ion (Cd or Zn) in the formation of ion-associates was calculated, and the drug concentration was determined indirectly.

ANALYTICAL DETERMINATION OF TADALAFIL IN PHARMACEUTICAL PREPARATIONS AND URINE SAMPLES

For analysis of Td, sampling was made by grinding up 25 tablets of Cialis and Snafi tablets then taking 0.55-155.75 µg. Urine samples were obtained from type II diabetic patients in Taif Area, Saudia Arabia (Ages from 45-55 years old) after 2 – 36 hours of taking dose. In all cases the tablets and urine samples were analyzed applying the above described procedure.

RESULTS AND DISCUSSION

The results of the elemental analysis (Table 2) of the produced solid ion-associates revealed that two drug cations form ion-ssociates with one [$Cd(SCN)_4$]$^{2-}$ or [$Zn(SCN)_4$]$^{2-}$. These results are comparable to the previously reported results.[12 – 14] Conductometric titrations of the investigated drug with $[Cd(SCN)_4]^{2-}$ or $[Zn(SCN)_4]^{2-}$ were performed to provide insight into the stoichiometric compositions of the ion-associates formed in solution. For all ion-associates, the characteristics curve-breaks are observed at a cation / anion mol ratio of about 2, confirming the formation of 2 : 1 (drug : X^{2-}) ion-associates. The obtainedresults were coincide with that of elemental analysis of the formed ion-associates. The optimum values of pH and ionic strength (Table 3) have been elucidated by determining the solubility of the ion-associates in HCl-NaOH solutions of different pH values and ionic strengths. The best were those exhibiting lowest solubility values.

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

Tadalafil HCl was determined precisely and accurately in aqueous solutions at their optimum conditions of pH and ionic strength (Table 4), in pharmaceutical preparations and urine samples using the present method. The results given in Table 4 reveal that recoveries were in the range 99.94 - 100.05 %, reflecting the high accuracy in addition to the high precision indicated by the very low values of the relative standard deviation. Generally, the

present method is as good as those reported before where, 0.32 - 160.65 µg mL^{-1} solutions of tadalafil using $[Cd(SCN)_4]^{2-}$ and $[Zn(SCN)_4]^{2-}$ weredetermined, respectively, which means that this method is applicable over a wider concentration range than that of our previously published method for tadalafil [4-5] in which tadalafil was determined in the ranges 64-117.81 and 0.53 – 139.23 µg mL^{-1}, respectively, Spectrophotometric methods[6-8] in which tadalafil was determined in the ranges 2-20, 2-20 and 1 – 7 µg mL^{-1}, respectively_ and HPLC method[9] in which tadalafil was determined in the range 10-150 µg $mL.^{-1}$ 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. It is clear from the results obtained for the pharmaceutical preparations (Table 4) that these excipiences do not interfere. In order to establish whether the proposed method exhibits any fixed or proportional bias, a simple linear regression [15] of observed drug concentration against the theoretical values (five points) was calculated. The student`s *t-test* [15] (at 95% confidence level) was applied to the slope of the regression line which showed that it did not differ significantly from the ideal value of unity. Hence, it can be concluded that there are no systematic differences between the determination and the true concentration over a wide range. The standard deviations (SD) can be considered satisfactory at least for the level of concentrations examined. Although the present method is more time consuming than some other methods, it exhibits fair sensitivity and accuracy. Moreover, the reproducibility of the results is superior to those obtained with other methods.

Table 1
Analytical Parameters for the Emission Measurement of Cd and Zn Using ICP-AES

Element	Wavelength (nm)	Order	Plasma position	DL (mg/L)	LDR (mg/L)	BEC (mg)	RSD x BEC (%)
Cd	214.43	105	0	0.005	0.05-300	0.4	1 x 1.0
Zn	206.20	109	0	0.01	0.1-1000	0.3	10 x 0.9

Note. DL, detection limit; LDR, linear dynamic range; BEC, background equivalent concentration; RSD, 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 tadalafil ion – associates

Drug	Ion-associate composition	m. p. °c	Molar ratio	Color	% Found (calculated) C	H	N	Metal (Zn or Cd)
Tadalafil	$(C_{27}H_{35}N_2O_7)_2[Zn(SCN)_4]$	358	2 : 1	white	53.77 (53.73)	5.44 (5.40)	8.69 (8.65)	5.09 (5.04)
	$(C_{27}H_{35}N_2O_7)_2[Cd(SCN)_4]$	298	2 : 1	white	51.89 (51.85)	5.24 (5.21)	8.37 (8.34)	8.41 (8.41)

Table 3
Solubility and solubility product of tadalafil ion-associates at their optimum conditions of pH and ionic strength (μ) values ate 25° C

Td- Ion – associate	pH	μ	p^s	pk_{sp}
$(C_{27}H_{35}N_2O_7)_2[Cd(SCN)_4]$	6.0	0.3	2.35	6.46
$(C_{27}H_{35}N_2O_7)_2[Zn(SCN)_4]$	4.0	0.2	4.44	12.72

p^s : -log solubility
pk_{sp} : -log solubility product

Table 4
Determination of Tadalafil in aqueous solutions, pharmaceutical Preparations and urine samples by ICP-AES and AAS

Sample	Amount taken (μg)	Mean recovery (%)	Mean RSD (%)
Using $[Zn(SCN)_4]^{2-}$ *			
Pure Td solution	**0.32 - 160.65**	**99.94**	**0. 5**
Snafi tablets [a]	**0.55 - 155.75**	**99.95**	**0.6**
Cialis tablets [b]	**0.55 - 155.75**	**99.95**	**0. 8**
Urine after 2 hs	**1.75 - 145.25**	**99.96**	**0.7**
Urine after 8 hs	**4.55 - 125.35**	**99.95**	**0.8**
Urine after 12 hs	**8.25 - 105.15**	**99.96**	**0.7**
Urine after 20 hs	**25.45 - 85.65**	**99.96**	**0.7**
Urine after 24 hs	**45.43 - 76.35**	**99.96**	**0.8**
Urine after 30 hs	**55.15 - 72.25**	**99.95**	**0.6**
Urine after 36 hs	**0.00**		
**Using $[Zn(SCN)_4]^{2-}$ ** **			
Pure Td solution	**0.32 - 160.65**	**99.97**	**0. 7**
Snafi tablets [a]	**0.55 - 155.75**	**99.98**	**0.8**
Cialis tablets [b]	**0.55 - 155.75**	**99.98**	**0. 9**
Urine after 2 hs	**1.75 - 145.25**	**99.97**	**0.7**
Urine after 8 hs	**4.55 - 125.35**	**99.97**	**0.8**
Urine after 12 hs	**8.25 - 105.15**	**99.98**	**0.8**
Urine after 20 hs	**25.45 - 85.65**	**99.97**	**0.7**
Urine after 24 hs	**45.43 - 76.35**	**99.98**	**0.8**
Urine after 30 hs	**55.15 - 72.25**	**99.97**	**0.7**
Urine after 36 hs	**0.00**		
Using $[Cd(NO_2)_6]^{3-}$ *			
Pure Td solution	**0.32 - 160.65**	**100.01**	**0.8**
Snafi tablets [a]	**0.55 - 155.75**	**100.02**	**0.9**
Cialis tablets [b]	**0.55 - 155.75**	**100.03**	**1.0**
Urine after 2 hs	**1.75 - 145.25**	**100.02**	**1.1**
Urine after 8 hs	**4.55 - 125.35**	**100.04**	**1.1**
Urine after 12 hs	**8.25 - 105.15**	**100.03**	**1.0**
Urine after 20 hs	**25.45 - 85.65**	**100.05**	**1.0**
Urine after 24 hs	**45.43 - 76.35**	**100.04**	**1.1**
Urine after 30 hs	**55.15 - 72.25**	**100.03**	**1.0**
Urine after 36 hs	**0.00**		

RSD : Relative Standard Deviation (five determinations) * By ICP-AES ** By AAS
[a] Eli Lilly and Company, USA
[b] Al- Qassim Pharmaceutical Plant, Saudi Arabia (SPIMACO)

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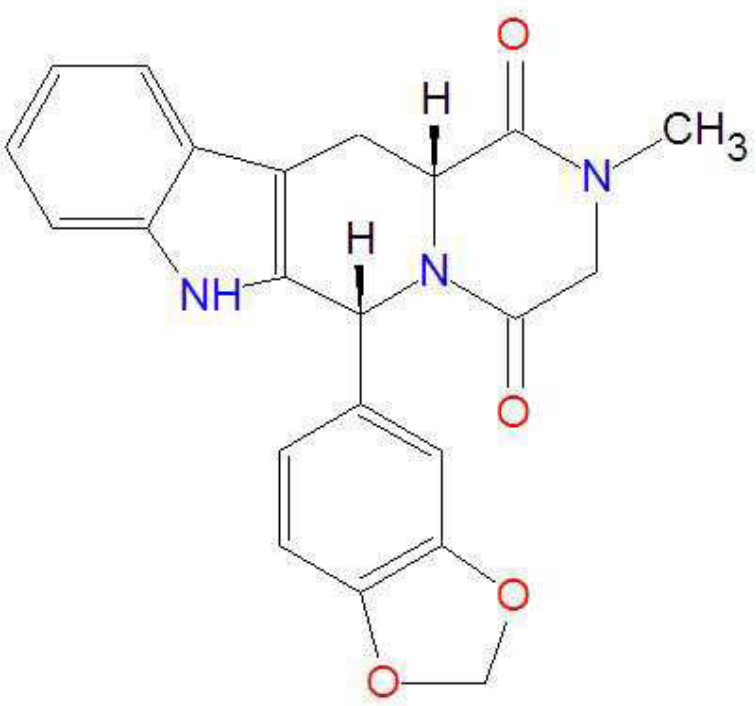

Figure (1)
Chemical structure of Tadalafil

CONCLUSION

The present method is as good as those reported before where, 0.32 - 160.65 µg mL^{-1} solution of tadalafil using $[Cd(SCN)_4]^{2-}$ and $[Zn(SCN)_4]^{2-}$ were determined, respectively, which means that this method is applicable over a wider concentration range than previously published methods. Tadalafil characterized by its long duration of action that reach 36 hours (as shown from the results of urine analysis at Table 4) compared to other products that last only for few hours. This long duration of action allows the patient to take tadalafil comfortably without time restriction for intercourse. For most patients, the recommended starting dose of tadalafil is 10 mg per day taken only when needed, just before sexual activity. The drug can help achieve an erection when sexual stimulation occurs. For once daily use without regard to sexual activity the recommended dose is 2.5 to 5 mg daily,allow 24 hours to pass between doses. Tadalafil does not interact with food and it can be used before or after meals.

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