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Analytical applications of atomic emission spectrometry for the microdetermination of sildenafil, tadalafil and vardenafil drugs employed in the erectile dysfunction therapy

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ABSTRACT

Ion – associate complexes of sildenafil, tadalafil and vardenafil hydrochlorides with potassium ferricyanide and ammonium reineckate were precipitated and the excess unreacted metal complex was determined. A new method using atomic emission spectrometry for the determination of the above drugs in pure solutions, in pharmaceutical preparations and urine of diabetic patients type 2 was given. The drugs can be determined by the affort method in the ranges 0.47 - 123.24, 0.53 - 139.23 and 0.52 – 136.37 μg mL^{-1} solutions of Sd, Td and Vd, respectively.

KEYWORDS

Atomic emission;
Ion-associate complexes;
Pharmaceutical analysis.

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].

Sildenafil; (Sd), tadalafil; (Td) and vardenafil; (Vd) are very important pharmaceutical compounds. Therefore, we found it important to prepare new ion-associates containing these drugs and to study and elucidate the chemical structures. Also the work presents a new rapid method for the

determination of these drugs after transformation into the ion-associates. The chemical structures of these drugs are shown in figure 1.

Sildenafil citrate (Sd cit);viagra is a potent and selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDES).

The activity of Sd cit for the treatment of male erectile dysfunction has been reported by several authors[3-8]. This drug should be administrated under instruction of doctors because its over dose might cause a series of side-effects[9,10].

Sd cit is chemically known as: 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo-[4,3-d]pyrimidin-5-yl)phenyl sulphonyl]-4-methylpiperazine citrate.

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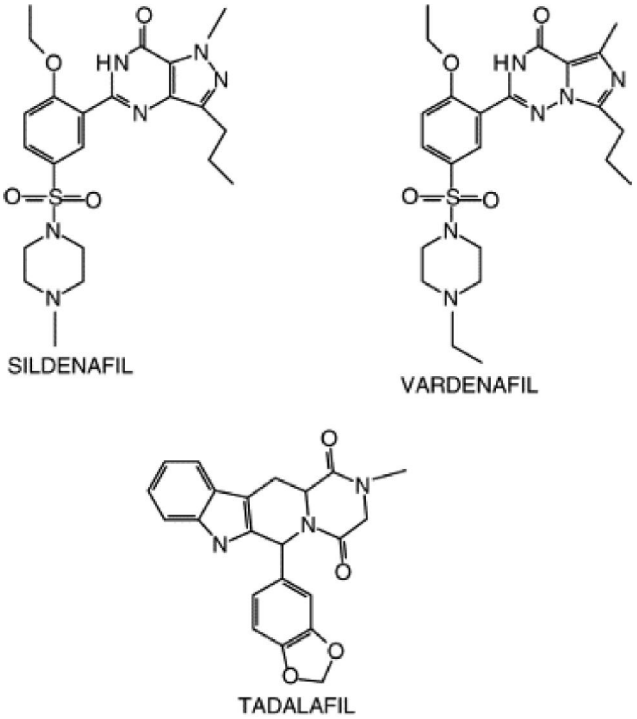

Figure 1 : Structures of sildenafil, vardenafil and tadalafil.

Tadalafil is a selective phosphodiesterase type 5 inhibitor, which is used to treat mild to severe ED in man. Drug testing is an integral part of pharmaceutical analysis and routine quality control monitoring of drug release characteristics.

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[12 ,22 :1,6]pyrido[3,4-b]indole-1,4-dione.

Vd is chemically known as: 1-[[3-(1,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-*f*][1,2,4]triazin-2-yl)-4-ethoxyphenyl]sulfonyl]-4-ethyl-monohydrochloride.

Studies in men with erectile dysfunction have shown that single doses of vardenafil 10 - 40 mg were rapidly absorbed following oral administration, with maximum plasma concentration reached in some men within 15 minutes[11,12]. Information from the patient diaries indicated that vardenafil increased the rate of successful intercourse compared with placebo, most patients receiving vardenafil indicated that their erections has improved after 12 weeks of treatment[13]. Clinical studies have demonstrated that Vd is a well-tolerated, effective and reliable treatment of ED and represents a valuable new therapy option for men with ED and their partners and many patients were returned to normal erectile function after treatment with vardenafil[14].

There is no official method for the determination of Sd cit in its formulations. Various reports have been described for the determination of Sd cit, those are accurate spectrochemical, chromatographic and electroanalytical methods[15-37]. Most of these methods are expensive, required careful control of conditions, suffer from lack of selectivity and time consuming [16,20, 21, 29, 35 -36].

To the best of our knowledge no report has been published on the analysis of tadalafil in pharmaceutical preparations. Also there is no official method for the determination of Vd in its formulations.

Few reports have been described for the determination of Vd, those are HPLC-MS[7], HPLC-coupled with liquid-liquid extraction[8], HPLC-with diode array detection[9],electrokinetic capillary chromatography[10] and electrochemical[11]. Since, most of these methods are expensive, required careful control of conditions, suffer from time-consuming extraction procedures[7-10], the use of simpler, faster, less expensive and sensitive method is required. The use of simpler, faster, less expensive and sensitive method is desirable.

Although, Direct Coupled Plasma-Atomic Emission Spectrometry (DCP-AES) is a rapid method and has a very low detection limit which can not be reached by most of the other methods. The present study includes new DCP-AES method for the determination of the investigated drugs. The method is based on the precipitating the ion-associates formed as a result of the combination of these drugs with an excess of $[Fe(CN)_6]^{3-}$ and ammonium reineckate; $[Cr(NH_3)_2(SCN)_4]^-$. 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. Sildenafil citrate (Asia Company for Pharmaceuticals,Sorya), Viagra tablets, containing 50 and 100 mg Sd cit per tablet were obtained from (Pfizer, USA). Tadalafil was

obtained from Eli Lilly and Company, USA. Cialis® tablet (containing 20 mg of tadalafil), manufactured by Eli Lilly and Company, USA, was purchased from local market. Vardenafil hydrochloride (Bayer Company, Leverkusen,Germany ;www.bayer.com), Levitra tablets, containing 20 mg Vd per tablet were obtained from a local pharmacy. potassium thiocyanate were from Aldrich(www.sigmaaldrich.com).

Apparatus

The pH of the solutions was measured using an Orion Research Model 701A digital pH-meter. Direct coupled plasma atomic emission measurements were carried out using ICPE- 9000 Shimadzu plasma atomic emission spectrometer. 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 solution of chromium was prepared by weighing 1.0 g of high purity sample of chromium shot, 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 was stable for approximately one year. Standard solution of iron was obtained from Aldrich.

Emission measurements

Analytical Parameters for the Measurement of Fe and Cr Using DCP-AES are listed in TABLE 1. The instrument was equally adequate for present purposes and was used according to availability. The atomic spectrometry was calibrated as in the previously reported work[36].

TABLE 1 : Analytical Parameters for the Measurement of Fe and Cr Using DCP-AES

Element	Wavelength (nm)	Order	Plasma position	DL (mg/L)	LDR (mg/L)	BEC (mg)	RSD x BEC(%)
Fe	248.30	90	0	0.01	0.1-1000	0.2	1 x 0.7
Cr	267.71	84	0	0.01	0.1-1000	0.4	7 x 0.7

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.

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[37] of the drugs with $[Fe(CN)_6]^{3-}$ and ammonium reineckate; $[Cr(NH_3)_2(SCN)_4]^-$ solutions.

Analytical determination of the drugs in aqueous solutions

Aliquots (0.025 - 6.5 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 $[Fe(CN)_6]^{3-}$ and $[Cr(NH_3)_2(SCN)_4]^-$ 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 AES. The consumed metal ion (Cr and Fe) in the formation of ion-associates was calculated, and the drug concentration was determined indirectly.

Analytical determination of drugs in pharmaceutical preparations and urine samples

For analysis of Sd, sampling was made by grinding up 8 tablets of Viagra tablets then taking 0.85-118.50 μg. For analysis of Td, sampling was mad by grinding up 15 tablets of Cialis tablets then taking 1.50-135.50 μg.

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In case of analysis Vd, sampling was made by grinding up 20 tablets of Levitra tablets then taking 0.95-132.50 µg of the tablets. Urine samples were obtained from type II diabetic patients in Taif Area, Saudia Arabia (Ages from 40-55 years old) after 2, 3 and 8 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 in all cases three drug cations form ion-ssociates with one $[Fe(CN)_6]^{3-}$ ion, while only drug cation combines with $[Cr(NH_3)_2(SCN)_4]^-$ to form a 1:1 ion associates. These results are comparable to the previously reported results[38 - 40].

Conductometric titrations of the investigated drugs with $[Fe(CN)_6]^{3-}$ and ammonium reineckate; $[Cr(NH_3)_2(SCN)_4]^-$ 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 (drug : X^{n-}) ion-associates as follow: in the case of the reinecka anion, the curve exhibits a sharp break at the 1:1 molecular ratio and in the case of ferricyanide anion the curve exhibits a sharp break at the 3:1 molecular ratio. The results obtained coincide with the elemental analysis of the precipitated ion-associates. The optimum pH and ionic strength values (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.

TABLE 2 : Elemental analysis, composition and some physical properties of the drug ion - associates

Drug	Ion-associate composition	m. p. ^{0}c	Molar ratio	Color	%Found (calculated)			
					C	H	N	Metal
Sildenafil	$(C_{22}H_{30}N6O_4S)_3[Fe(CN)_6]$	326	3 : 1	brown	52.83 (52.88)	5.48 (5.51)	20.52 (20.56)	3.41Fe (3.43)
	$(C_{22}H_{30}N6O_4S)[Cr(NH_3)_2(SCN)_4]$	344	1 : 1	pink	39.35 (39.39)	4.49 (4.55)	21.17 (21.21)	6.53Cr (6.56)
Tadalafil	$(C_{27}H_{35}N_2O_7)_3[Fe(CN)_6]$	376	3 : 1	brown	60.88 (61.09)	6.11 (6.14)	9.81 (9.83)	3.25Fe (3.28)
	$(C_{27}H_{35}N_2O_7)[Cr(NH_3)_2(SCN)_4]$	312	1 : 1	pink	45.49 (45.53)	5.00 (5.02)	13.68 (13.70)	6.28Cr (6.36)
Vardenafil	$(C_{23}H_{32}N_6O_4S)_3[Fe(CN)_6]$	278	3 : 1	brown	53.66 (53.70)	5.68 (5.73)	19.96 (20.05)	3.31Fe (3.34)
	$(C_{23}H_{32}N_6O_4S)[Cr(NH_3)_2(SCN)_4]$	262	1 : 1	pink	40.16 (40.19)	4.68 (4.71)	20.79 (20.84)	6.42Cr (6.45)

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

Drug	Ion – associate	p^H	µ	p^s	pk_{sp}
Sd	$(C_{22}H_{30}N6O_4S)_3[Fe(CN)_6]$	3.0	0.5	2.55	8.80
	$(C_{22}H_{30}N6O_4S)[Cr(NH_3)_2(SCN)_4]$	2.0	0.3	4.20	8.39
Td	$(C_{27}H_{35}N_2O_7)_3[Fe(CN)_6]$	5.0	0.2	2.30	7.76
	$(C_{27}H_{35}N_2O_7)[Cr(NH_3)_2(SCN)_4]$	3.0	0.3	4.19	8.39
Vd	$(C_{23}H_{32}N_6O_4S)_3[Fe(CN)_6]$	4.0	0.6	2.28	7.07
	$(C_{23}H_{32}N_6O_4S)[Cr(NH_3)_2(SCN)_4]$	3.0	0.2	4.25	8.51

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

Anlytical determination of drugs in aqueous solutions, pharmaceutical preparations and urine samples

Sildenafil HCl, tadalafil HCl and vardenafil HCl were 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 99.96 - 101.18 % and 99.76 – 100.12 %, 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 0.47 - 123.24, 0.53 - 139.23

TABLE 4 : Determination of the investigated drugs in aqueous solutions, pharmaceutical preparations and urine samples by AES

Sample	Taken (μ g)	Mean Recovery (%)	Mean RSD (%)
* Using [Fe $(CN)_6$]$^{3-}$	0.47 – 123.24	99.96	0.8
Sildenafil solution	0.85 - 118.50	100.04	0.7
Viagra tablets [a]	0.68 - 92.18	100.02	0.8
Urine after 2hs	2.35 - 103.15	100.08	0.9
Urine after 3hs	112.14 - 118.22	100.06	1.1
Urine after 8hs			
Tadalafil solution	0.53 – 139.23	101.06	0.8
Cialis tablets [b]	1.50 - 135.50	101.10	1.1
Urine after 2hs	2.15 - 128.12	101.06	1.2
Urine after 3hs	6.25 - 116.35	100.07	0.7
Urine after 8hs	0.00		
Vardenafil solution	0.52 – 136.37	101.12	0.6
Levitra tablets [c]	0.95 - 132.50	101.18	1.2
Urine after 2hs	3.28 - 124.16	101.11	1.1
Urine after 3hs	8.15 - 117.25	100.12	0.8
Urine after 8hs	0.00		
** Using [$Cr(NH_3)_2(SCN)_4$]$^{1-}$	0.47 – 123.24	99.76	1.1
Sildenafil solution	0.85 - 118.50	100.02	1.2
Viagra tablets [a]	0.68 - 92.18	100.01	0.8
Urine after 2hs	2.35 - 103.15	100.03	0.7
Urine after 3hs	112.14 - 118.22	100.04	0.9
Urine after 8hs			
Tadalafil solution	0.53 – 139.23	100.06	1.1
Cialis tablets [b]	1.50 - 135.50	100.02	1.0
Urine after 2hs	2.15 - 128.12	101.03	0.8
Urine after 3hs	6.25 - 116.35	100.08	0.9
Urine after 8hs	0.00		
Vardenafil solution	0.52 – 136.37	100.12	0.8
Levitra tablets [c]	0.95 - 132.50	100.05	0.9
Urine after 2hs	3.28 - 124.16	101.00	1.0
Urine after 3hs	8.15 - 117.25	100.08	0.9
Urine after 8hs	0.00		

RSD : Relative Standard Deviation (six determinations); [a]Pfizer [b]Eli Lilly and Company, USA. [c]Bayer Company, Leverkusen,Germany.

and 0.52 – 136.37 μg mL^{-1} solutions of Sd, Td and Vd using $[Fe(CN)_6]^{3-}$ and ammonium reineckate; $[Cr(NH_3)_2(SCN)_4]^-$ were determined, respectively, which means that this method is applicable over wider concentration ranges than previously published methods for Sd[14,17,20] in which Sd was determined using micro-bore liquid chromatography by Panderi and Poulou, derivative spectro-photometry by El-Gindy et al. and ratio spectra derivative spectro-photometry by Nevin Erk in the ranges 5-20, 4-20 and 8-36 μg mL^{-1}, respectively. For Td[29] in which Td was determined using HPLC by Erturk et al. in the range 1.0 – 11 μg mL^{-1}. In case of Vd[35] in which Vd was determined using HPLC by Gumieniczek and Hopkala in the range 0.1 – 0.5 mg 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[41] of observed drug concentration against the theoretical values (five points) was calculated. The student's *t-test*[41] (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.

CONCLUSION

The present method is as good as those reported before where, 0.47 - 123.24, 0.53 - 139.23 and 0.52 – 136.37 μg mL^{-1} solutions of Sd, Td and Vd using $[Fe(CN)_6]^{3-}$ and ammonium reineckate; $[Cr(NH_3)_2(SCN)_4]^-$ were determined, respectively, which means that this method is applicable over wider concentration ranges than previously published methods

For most patients, the recommended dose of Sd is 50 mg taken, as needed, approximately 1 hour before sexual activity. However, Viagra may be taken anywhere from 4 hours to 0.5 hour before sexual activity. Based on effectiveness and toleration, the dose may be increased to a maximum recommended dose of 100 mg or decreased to 25 mg. The maximum recommended dosing frequency is once per day.

For most individuals, the recommended starting dose of Td is 10 mg per day taken before sexual activity. Depending on the adequacy of the response or side effects, the dose may be increased to 20 mg or decreased to 5 mg a day. The effect of Td may last up to

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36 hours. Individuals who are taking medications that increase the blood levels of Td should not exceed a total dose of 10 mg in 72 hours. For once daily use without regard to sexual activity the recommended dose is 2.5 to 5 mg daily. Td may be taken with or without food since food does not affect its absorption from the intestine.

With respect to Vd the normal starting dose is 10 mg (roughly equivalent to 50 mg of Sd) it should be taken 1 to 2 hours prior to sexual activity, with a maximum dose frequency of once per day. Vd should not be used by men taking nitrate medications, because combining them with Vd might provoke potentially life-threatening hypotension (low blood pressure).

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