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Analytical utility of atomic emission spectrometry for the determination of sildenafil citrate (Viagra) in pharmaceutical formulations

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ABSTRACT

Ion-associate complexes of sildenafil citrate; Sd cit (viagra) with (Cd(II) and Zn(II)) thiocyanates, potassium ferricyanide 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. The solubility products were thus calculated at different temperatures and the thermodynamic parameters ΔH, ΔG and ΔS were calculated. A new accurate and precise method based on direct coupled plasma atomic emission spectrometry for the determination of sildenafil citrate (0.75-85.32 μg/ml) in pure solutions and pharmaceutical preparations is given.

KEYWORDS

Sildenafil citrate;
Pharmaceutical analysis;
Ion-associate complexes;
Atomic emission spectrometry.

INTRODUCTION

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[1-6]. This drug should be administrated under instruction of doctors because its over dose might cause a series of side-effects[7, 8].

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 su-lphonyl]-4-methylpiperazine citrate (Figure 1).

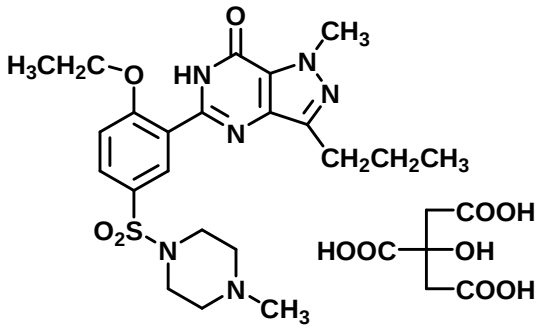

Figure 1 : Chemical structure of Sildenafil citrate

Because of the pharmaceutical properties of Sd cit we found it important to prepare new ion associates containing sildenafil and to study and elucidate their chemical structures to be applied to the analysis

of Sd cit. 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[9-30]. Most of these mehods are expensive, required careful control of conditions, suffer from lack of selectivity and time consuming[10, 14, 15, 23, 29, 30].

Although direct coupled plasma atomic emission Spectrometry (DCP-AES) is a rapid method and has very low detection limits which can not be reached by most of the above mentioned methods, it has not been applied yet to the determination of Sd cit. The present work includes a new DCP-AES method for the determination of Sd cit. The method is based on precipitation of the ion associates formed from the reaction of Sd cit with $[Cd(SCN)_4]^{2-}$, $[Zn(SCN)_4]^{2-}$, ammonium reineckate, $[Cr(NH_3)_2(SCN)_4]^-$, or $[Fe(CN)_6]^{3-}$. The metal ion content present in saturated solutions of these ion associates is determined employing DCP-AES and is used to calculate the concentration of Sd cit.

DCP-AES is well suited for this type of determination because of its accuracy, precision, sensitivity, and freedom from interference.

EXPERIMENTAL

Reagents

Double-distilled water and analytical grade reagents were used to prepare all solutions. Sildenafil citrate (Asia Company for Pharmaceuticals,Sorya),ammonium reineckate, potassium ferricyanide and zinc acetate were Aldrich products, cadmium nitrate (BDH), Caverta tablets, containing 100 mg Sd cit per tablet were obtained from (Ranbaxy Laboratories, India), Vega tablets, containing 50 mg Sd cit per tablet were obtained from (Asia Company for Pharmaceuticals,Sorya) and Edegra tablets, containing 50 mg Sd cit per tablet were obtained from (Sun Pharmaceuticals Industries Ltd.).

Apparatus

The pH of solutions was measured using an Orion (Cambridge, MA, USA) digital pH meter. Direct current plasma atomic emission spectrometry for the determination of metal ion is carried out using a Beckman Spectra Span III 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 ion associates

The ion associates were prepared by mixing solutions containing 1 x 10^{-3} mol of Cd(II), or Zn(II) with a solution containing 4 x 10^{-3} mol of potassium thiocyanate and the requisite amount of Sd cit. Potassium ferricyanide and ammonium reineckate 1 x 10^{-3} mol of the solution was mixed with the calculated amount of Sd cit. 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

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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.

Effect of temperature

The effect of temperature on the solubility of ion associates and the heat of the solution of ion associates were studied by preparing a suspension of the ion associate in solutions at the optimum pH and ionic strength values at different temperatures (25, 35, 45, and 60°C). The metal ion content present in the form of soluble complex ion is measured using DCP-AES and the heat of the solution of the ion associates was determined applying the Van't Hoff isochore relation; thus:

$$\log S = -\Delta H / 2.303RT + \text{constant}, \quad (1)$$

Where H is the heat of solution KJ mol^{-1} ; R is the universal gas constant, 8.3 J mol^{-1} K^{-1} ; and T is the absolute temperature in K. Thus,

a plot of log S against $1/T$ is a straight line with a slope equal to $(-\Delta H / 2.303 R)$ from which ΔH is calculated.

Gibb's free energy change (ΔG) and the entropy change

(ΔS) are calculated using Eqs. (2) and (3), respectively,

$$\Delta G = -RT \ln K_{sp}, \quad (2)$$

Where K_{sp} is the solubility product of ion associate and

$$\Delta G = \Delta H - T\Delta S \quad (3)$$

Preparation of standard solutions

Standard solutions of divalent cadmium, chromium and zinc are prepared by weighing 1.0 g of a high-purity sample (cadmium shot, cobalt powder, 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 DCP-AES

Under the recommended conditions, calibration graphs were constructed of aqueous standards of cadmium(II), chromium(III), zinc(II) and Fe(III) in 1 M $HN0_3$ by performing triplicate measurements using solutions containing 0, 10, 20, and 50 ppm analyte concentrations as previously reported[31, 32]. 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 Fe(III) are listed in TABLE 1.

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

Wave length	Element (nm)	Order	Plasma position	DL (mg/L)	LDR (mg/L)	BEC (mg)	RSDx BEC (%)
Cd	214.43	105	0	0.005	0.05-300	0.4	1x1.0
Fe	248.30	90	0	0.01	0.1-1000	0.2	1x0.7
Cr	267.71	84	0	0.01	0.1-1000	0.4	7x0.7
Zn	206.20	109	0	0.01	0.1-1000	0.3	10x0.9

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.

Conductimetric measurements

The stoichiometry of the ion associates was elucidated by conductimetric titration of Sd cit with the metal complex solutions.

Analytical determination of Sd cit in aqueous solutions

Aliquots (0.04 - 4.5 ml) of 0.001 M Sd 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) or Zn(II)) thiocyanate, ferricyanide, 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 Sd cit in pharmaceutical preparations

The sildenafil-containing pharmaceutical prepara-

tions (Caverta, Vega and Edegra tablets) were successfully assayed using the present method. Sampling were made by grinding (8, 12 and 20 tablets) then taking 1.50-82.65, 2.50-74.25 and 2.65-83.50 µg / ml of the Caverta, Vega and Edegra tablets, respectively at the optimum condition solution and the tablets were analyzed applying the above mentioned procedure.

RESULTS AND DISCUSSION

Composition and structure of ion associates

The results of elemental analysis (TABLE 2) of the produced solid ion associates reveal that two sildenafilinium cations form ion associates with one $[M(SCN)_4]^{2-}$ and three $[Fe(CN)_6]$,$^{3-}$ while only one Sd combines with $[Cr(NH_3)_2(SCN)_4]^-$ to form a 1:1 ion associate. These results are comparable to the previously reported results[31-33].

Conductimetric titrations of the investigated inorganic complexes with Sd cit 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 ([Sd] / $[x]^{n-}$) of about 2, confirming the formation of 2:1 (Sd : 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 and in 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 associate.

Effect of pH on the formation 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.,

$$(Sd)_2[M(SCN)_4] \rightleftarrows 2Sd^+ + [M(SCN)_4]^{2-}$$

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 sildenafilinium 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.

TABLE 3 : Solubility and Solubility Product Values of Sd cit Ion Associates at Optimum pH and 25°C

Ion associate	pH	pS	pK_{sp}	pk_{sp}
$(Sd)_2[Cd(SCN)_4]$	4.0	2.99	8.37	7.97
$(Sd)_2[Zn(SCN)_4]$	3.0	2.67	7.42	9.35
$(Sd)_3[Fe(CN)_6]$	2.0	2.28	7.70	6.77
$(Sd)[Cr(NH_3)_2(SCN)_4]$	3.0	4.20	8.40	7.08
				8.10

NoNote. pS, -log solubility. pksp, -log solubility product.

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

TABLE 2 : Elemental Analysis, Composition, and Some Physical Properties of Sildenafil citrate Ion Associates

Ion-associate composition	*m.p* °C	Molar ratio	Color	% found (calculated)				
				C	H	N	S	Metal
$(C_{22}H_{30}N6O_4S)_2[Cd(SCN)_4]$	236	2:1	White	44.56 (44.60)	4.64 (4.68)	17.33 (17.39)	14.85 (15.02)	8.69 (8.72)
$(C_{22}H_{30}N6O_4S)_2[Zn(SCN)_4]$	276	2:1	White	46.25 (46.32)	4.82 (4.92)	11.24 (11.36)	15.42 (15.63)	5.24 (5.36)
$(C_{22}H_{30}N6O_4S)[Cr(NH_3)_2(SCN)_4]$	298	1:1	Pink	39.39 (39.45)	4.55 (4.62)	21.21 (21.28)	20.20 (20.34)	6.56 (6.64)
$(C_{22}H_{30}N6O_4S)_3[Fe(CN)_6]$	242	3:1	White	52.88 (52.94)	5.51 (5.58)	20.56 (20.72)	5.88 (5.39)	3.43 (3.52)

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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 4). 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 (4). The results indicate that the present ion associates are so sparingly soluble that Sd cit can be determined accurately and precisely by the indirect method through precipitation of its ion associates with [Cd(II) and Zn(II)] thiocyanate complexes, potassium ferricyanide and ammonium reineckate.

TABLE 4 : Solubility and Solubility Product Values at 25°C of Sd cit Ion Associates at Their Optimum pH and Ionic Strength (μ) Values

Ion Associate	pH	μ	pS	pK_{sp}
(Sd)2[$Cd(SCN)_4$]	4.0	0.3	3.25	9.15
$(Sd)_2[Zn(SCN)_4]$	3.0	0.4	3.14	8.82
$(Sd)_3[Fe(CN)_6]$	2.0	0.6	2.62	9.05
$(Sd)[Cr(NH_3)_2(SCN)_4]$	3.0	0.7	4.26	8.52

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

Effect of temperature on the solubility of ion associates

The solubility of ion associates was investigated at different temperatures (25, 35, 45, and 60°C) and the heat of solution (ΔH), Gibb's free energy change (ΔG), and the entropy change (ΔS) have been calculated (TABLE 5). The results show that Sd cit is better determined at 25°C than at higher temperatures, providing the optimum conditions of pH and ionic strength. This is because increasing temperature increases the solubility where the process of dissolution of the precipitates is endothermic because the lattice energy is usually greater than the solvation energy and hence the stability of ion associates decreases.

Gibb's free energy ΔG increases when the solubility of ion associates is decreased.

Analytical determination of Sd cit in aqueous solutions and tablets

Sd cit was determined precisely and accurately in aqueous solutions and in the pharmaceutical preparation (Caverta, Vega and Edegra tablets) using the present method. The results given in (TABLE 6) reveal that for ammonium reineckate the recovery is 100.12 %, reflecting a high accuracy which in addition to the high precision indicated by very low values of relative stan-

TABLE 5 : Solubility (S), Solubility Product (K_{sp}), and Some Thermodynamic Functions of Sildenafil Ion Associate at Different Temperatures

t °C	S (g mol/liter)	K_{sp}	ΔG (kJ mol-')	ΔS (kJ mol^{-1})	ΔH (kJ mol^{-1})	S (g mol/liter)	K_{sp}	ΔG (kJ mol^{-1})	Δ S (kJ mol^{-1})	ΔH (kJ mol^{-1})
	$(Sd)_2[Cd(SCN)_4]$					$(Sd)[Cr(NH_3)_2(SCN)_4]$				
25	5.60×10^{-4}	7.02×10^{-10}	52.13	8.89	2.7×10^3	5.50×10^{-5}	3.02×10^{-9}	48.52	6.68	2.0×10^3
35	4.20×10^{-4}	2.96×10^{-10}	19.87	8.70		6.32×10^{-5}	3.99×10^{-9}	24.71	6.54	
45	5.10×10^{-4}	5.30×10^{-10}	56.37	8.31		7.18×10^{-5}	5.16×10^{-9}	50.36	6.25	
60	8.20×10^{-4}	2.20×10^{-9}	55.09	7.94		8.31×10^{-5}	6.90×10^{-9}	51.93	5.97	
	$(Sd)_2[Zn(SCN)_4]$					$(Sd)_3[Fe(CN)_6]$				
25	7.20×10^{-4}	1.49×10^{-9}	50.27	3.96	1.23×10^3	2.40×10^{-3}	8.95×10^{-10}	51.53	15.26	4.6×10^3
35	6.12×10^{-4}	9.16×10^{-10}	53.20	3.82		2.88×10^{-3}	1.85×10^{-9}	51.40	14.77	
45	6.43×10^{-4}	1.06×10^{-9}	54.54	3.97		3.96×10^{-3}	6.64×10^{-9}	49.70	14.31	
60	5.72×10^{-4}	7.48×10^{-10}	58.07	3.52		4.32×10^{-3}	9.40×10^{-9}	51.08	13.66	

TABLE 6 : Analytical Determination of Sildenafil Citrate in Aqueous Solution and in Caverta, Vega and Edegra Tablets by DCP-AES

Sample	Amount taken (µg)	Mean recovery (%)	Mean RSD (%)
$[Cd(SCN)_4]^{2-}$			
Pure Sd cit solution	0.75-85.32	97.76	0.87
Caverta Tablets[(a)]	1.50-82.65	96.82	1.03
Vega tablets[(b)]	2.50-74.25	97.75	1.06
Edegra tablets[(c)]	2.65-83.50	97.74	1.02
$[Zn(SCN)_4]^{2-}$			
Pure Sd cit solution	0.75-85.32	98.88	1.06
Caverta Tablets[(a)]	1.50-82.65	97.86	1.09
Vega tablets[(b)]	2.50-74.25	97.83	1.07
Edegra tablets[(c)]	2.65-83.50	97.82	1.08
$[Cr(NH_3)_2(SCN)_4]^-$			
Pure Sd cit solution	0.75-85.32	100.12	0.45
Caverta Tablets[(a)]	1.50-82.65	100.06	0.65
Vega tablets[(b)]	2.50-74.25	100.10	0.68
Edegra tablets[(c)]	2.65-83.50	100.03	0.66
$[Fe(CN)_6]^{3-}$			
Pure Sd cit solution	0.75-85.32	97.73	1.13
Caverta Tablets[(a)]	1.50-82.65	96.58	1.15
Vega tablets[(b)]	2.50-74.25	96.76	1.14
Edegra tablets[(c)]	2.65-83.50	96.74	1.12

Note. RSD, relative standard deviation (six determinations). (a) Ranbaxy Laboratories, India. (b) Asia Company for Pharmaceuticals,Sorya. (c) Sun Pharmaceuticals Industries Ltd.

dard deviations. For (Cd and Zn) thiocyanates and ferricyanide the recovery range is between 96.58 and 98.88_% -less accurate than that for ammonium reineckate.

Generally, the present method is applicable over a wider concentration range; (0.75 - 85.32 µg/ml) than that of[10, 14, 30] where 66.4 - 332, (1.25-50, 1.25- 60) and 0.8-80 µg/ml solution of Sd cit 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 6) that these excipiences do not interfere.

TABLE 7 : Linear regression analysis for Sildenafil citrate using thiocyanates of [Cd and Zn], ammonium reineckate and potassium ferricyanide.

Parameters	Thiocyanates of		Ammonium	Potassium
	Cd	Zn	reineckate	ferricyanide
Optimum concentration range (µg / ml)	0.75-85.32	0.75-85.32	0.75-85.32	0.75-85.32
Shift or intercept of the regression line [a]	0.027	0.031	0.033	0.032
Slope of regression line	0.99878	1.0052	1.0065	1.0073
Student's / (2.31)[b]	2.14	2.16	2.07	2.09
Range of error (%)	99.8 +1.3	100.0 +1.2	100.0 +1.5	100.0 +1.3

(a) Observed versus theoretical. (b) Tabulated 95% confidence limit (for slope).

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[25, 29, 30]. 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[34] of observed drug concentration against the theoritical values (five points) was calculated. Student, s *t*-test[35] (at 95% confidence level) was applied to slope of the regression line (TABLE 7) 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.

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 that obtained from other methods.

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