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Analytical utilities of atomic absorption and 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;(Sd), tadalafil;(Td) and vardenafil;(Vd) hydrochlorides with iodomercurate(II),$[HgI_4]^{-2}$ and iodo-bismuthate(III), $[BiI_4]^{-1}$ were precipitated and the excess unreacted mercury or bismuth complex was determined. A new method using atomic emission and atomic absorption spectrometry for the determination of the above drugs in pure solutions and in pharmaceutical preparations is given. The drugs can be determined by the affort method in the ranges 0.948 - 56.88, 0.998 - 59.88 and 0.976 – 58.56 μg. mL^{-1} solutions of Sd, Td and Vd, respectively.

KEYWORDS

Atomic emission;
Atomic absorption;
Sildenafil;
Tadalafil and vardenafil;
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, tadalafil and vardenafil 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 present a new rapid method for the determination of these drugs after transformation into the ion-associates.

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.

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-hexahydro-2-me-

thyl-,(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.

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]sulfo-nyl]-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 mehods 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.

The determination of tadalafil in pharmaceutical formulations has been achieved by high-performance liquid chromatography (HPLC)[37-38] and capillary electrophoresis with UV detection[39-40]. Two liquid chromatography–electrospray ionization mass spectrometry methods were used for the simultaneous determination of undeclared PDE5 inhibitors, sildenafil, vardenafil and tadalafil, in dietary supplements[36,41,42]. As the concentrations of tadalafil prepared for analysis from pharmaceutical preparations and dietary supplements are much higher than those in plasma, these reported methods in general can not be used directly to measure plasma levels of tadalafil, due to inadequate sensitivity or interference by endogenous components in plasma. Recently, an HPLC coupled with electrospray ionization tandem mass spectrometry (LC–MS/MS) method was developed and validated for the determination of tadalafil in 250 µl of plasma[43]. Another LC–MS/MS method, without given full validation details, was also reported to measure plasma levels of tadalafil in a drug–drug interaction study[44]. Although these methods offer high-throughput analysis, they are not readily accessible because of the use of relative expensive tandem mass spectrometry.

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[45], HPLC-coupled with liquid-liquid extraction[46], HPLC-with diode array detec-tion[47], electrokinetic capillary chromatography[48] and electro-chemical[49]. Since, most of these mehods are expensive, required careful control of conditions, suffer from time-consuming extraction procedures[45-48]. The use of simpler, faster, less expensive and sensitive method is desirable.

Although, Direct Coupled Plasma-Atomic Emission Spectrometry (DCP-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 DCP-AES and AAS methods 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 $[HgI_4]^{-2}$ and $[BiI_4]^{-1}$. 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.

EXPERIMENTAL

Reagent and materials

Doubly-distilled water and analytical grade reagents were used in the preparation of all solutions. Sildenafil citrate (Asia Company for Pharmaceuticals, Sorya), 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.). 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 10 mg Vd per tablet were obtained from local pharmacy. Mercury(II) iodide, potassium iodide and mercury atomic absorption standard solution 1000 ìg mL^{-1} of Hg in 10 % HNO_3 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 a Beckman spectra span III emission spectrometer and atomic absorption measurements were made on Hitachi atomic absorption Z-6100 polarized Zeeman 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

The standard solution of Bi(III) was prepared by dissolving 1.0 g of bismuth metal in a minimum volume of (1+1) HNO_3. Dilute to 1-liter with 2% (v/v) HNO_3. The 1000 μg mL^{-1} Bi solutions was stored in a plastic bottle which had been presoaked in dilute HNO_3. The solution was stable for approximately one year.

Emission and absorption measurements

Using AES the bismuth was measured at wavelength 306.8 nm, order 87, plasma position 0.0, detection limit 0.003 μg mL^{-1}, linear dynamic ranges 0.03-100 μg mL^{-1}, background equivalent concen-tration 0.1 mg, entrance slits 50 x 300 μm and exit slits 100 x 300 μm. Using AAS the Hg (II) was measured at wavelength 253.7 nm, slit 0.7 nm, relative noise 4.2, detection limit 0.28 μg mL^{-1}, linear dynamic range 0.01-100 μg mL^{-1}, lamb current 5mA, and integration time 3s, the flame used was the acetylene–air mixture. 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[50,51].

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 h 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[52] of the drugs with $[BiI_4]^{-1}$ or $[HgI_4]^{-2}$ solutions.

Analytical determination of the drugs in aqueous solutions

Aliquots (0.05 - 3.0 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 $[BiI_4]^{-1}$ or $[HgI_4]^{-2}$ was added and the volume was coTdleted 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 or AAS. The consumed metal ion (Bi or Hg) in the formation of ion-associates was calculated, and the drug concentration was determined indirectly.

Analytical determination of drugs in pharmaceutical preparations

The sildenafil-containing pharmaceutical preparations (Caverta, Vega and Edegra tablets) were successfully assayed using the present method. Sampling were made by grinding (10, 12 and 15 tablets) then taking 2.25-51.32, 2.65-52.14 and 2.15-50.36 μg.mL^{-1} of the Caverta, Vega and Edegra tablets, respectively at the optimum condition solution.

For analysis of Vd, the vardenafil-containing pharmaceutical preparation (Levitra tablets) were successfully assayed using the present method. Sampling were

Full Paper

made by grinding (12 tablets) then taking 2.15-53.22 $\mu g.mL^{-1}$ of the Levitra tablets at the optimum condition solution and the tablets were analyzed applying the above mentioned procedure. In case of Td, sampling was made by grinding up 10 tablets then taking 1.75-55.50 $\mu g.mL^{-1}$ of Cialis tablets at the optimum condition solution. In all cases the tablets were analyzed applying the above described procedure.

RESULTS AND DISCUSSION

The results of the elemental analysis (TABLE 1) of the produced solid ion-associates revealed that in case of Bi one drug cation form ion-ssociates with one $[BiI_4]^{-1}$ and in case of Hg two drug cations form ion-ssociates with one $[HgI_4]^{-2}$ ion. These results are comparable to the previously reported results[53-55].

TABLE 1 : Elemental analysis, composition and some physical properties of the drug ion – associates

Drug	Ion-associate coTdosition	m. p. ^{0}c	Molar Ratio	Color	% Found (calculated)			
					C	H	N	Metal (Bi or Hg)
Sildenafil	$(C_{22}H_{30}N6O_4S)$ $[BiI_4]$	345	1 : 1	orange	22.13 (22.17)	2.55 (2.52)	7.09 (7.05)	17.58 (17.54)
	$(C_{22}H_{30}N6O_4S)_2$ $[HgI_4]$	364	2 : 1	yellow	31.91 (31.87)	3.65 (3.62)	10.17 (10.14)	12.16 (12.11)
Tadalafil	$(C_{27}H_{35}N_2O_7)$ $[BiI_4]$	315	1 : 1	orange	26.67 (26.64)	2.91 (2.88)	2.33 (2.30)	17.22 (17.18)
	$(C_{27}H_{35}N_2O_7)_2$ $[HgI_4]$	294	2 : 1	yellow	38.02 (37.97)	4.15 (4.10)	3.33 (3.28)	11.81 (11.75)
Vardenafil	$(C_{23}H_{32}N_6O_4S)$ $[BiI_4]$	246	1 : 1	orange	22.94 (22.90)	2.71 (2.66)	7.02 (6.97)	17.37 (17.34)
	$(C_{23}H_{32}N_6O_4S)_2$ $[HgI_4]$	224	2 : 1	yellow	32.81 (32.76)	3.84 (3.80)	10.01 (9.97)	11.95 (11.91)

Conductometric titrations of the investigated drugs with $[BiI_4]^{-1}$ and $[HgI_4]^{-2}$ were performed to provide insight into the stoichiometric compositions of the ion-associates formed in solution. With all ion-associates, the characteristics curve-breaks are observed at a cation / anion mol ratio of about 2 in case of Hg confirming the formation of 2:1 (drug : X^{2-}) but it was of about 1 in case of Bi confirming the formation of 1:1 (drug : X^{-1}) ion-associates. The results obtained coincide with the elemental analysis of the precipitated ion-associates. The optimum pH and ionic strength values (TABLE 2) 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 drugs in aqueous solutions and pharmaceutical preparations

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 2) and in pharmaceutical preparations using the present method. The results given in TABLE 3 reveal that recoveries were in the range 99.96 - 101.35 % and 98.88 - 101.14 %, 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, 0.948 - 56.88, 0.998 - 59.88 and 0.976 – 58.56 $\mu g.mL^{-1}$ solutions of Sd, Td and Vd using $[BiI_4]^{-1}$ and $[HgI_4]^{-2}$ were determined, respectively, which means that this method is applicable over a wider concentration range than previously published methods for Sd[16,20] by Liu et al. and Dinesh et al. in which Sd

TABLE 2 : Solubility and solubility product of the ion-associates at their optimum conditions of pH and ionic strength (μ) values ate 25 ^{o}C

Ion – associate	pH	μ	p^s	pk_{sp}
Sildenafil -iodobismuthate	3.0	0.3	13.95	7.89
Tadalafil - iodobismuthate	2.0	0.2	12.97	25.95
Vardenafil-iodobismuthate	4.0	0.4	11.96	23.92
Sildenafil –iodomercurate	5.0	0.3	10.95	32.25
Tadalafil -iodomercurate	6.0	0.5	9.88	29.04
Vardenafil-iodomercurate	5.0	0.2	10.85	34.06

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

TABLE 3 : Determination of the investigated drugs in aqueous solutions and in pharmaceutical preparations by AES and AAS

Sample	Taken (μg)	Mean recovery (%)	Mean RSD (%)
Using $[BiI_4]^{-1}$*			
Sildenafil solution	0.948 - 56.88	99.96	1.3
Caverta tablets[(a)]	2.25 - 51.32	101.05	1.2
Vega tablets[(b)]	2.65 - 52.14	101.07	1.1
Edegra tablets[(c)]	2.15 – 50.36	101.04	1.2
Tadalafil solution	0.998 - 59.88	101.12	0.8
Cialis tablets[(d)]	1.75 - 55.50	101.08	1.4
Vardenafil solution	0.976 - 58.56	101.35	0.3
Levetra tablets[(e)]	2.15 - 53.22	101.14	0.5
Using $[HgI_4]^{-2}$,*,**			
Sildenafil solution	0.948 - 56.88	98.88	1.2
Caverta tablets[(a)]	2.25 - 51.32	98.96	1.0
Vega tablets[(b)]	2.65 - 52.14	100.03	1.1
Edegra tablets[(c)]	2.15 – 50.36	100.02	1.2
Tadalafil solution	0.998 - 59.88	101.04	0.6
Cialis tablets[(d)]	1.75 - 55.50	100.02	1.1
Vardenafil solution	0.976 - 58.56	101.14	0.4
Levetra tablets[(e)]	2.15 - 53.22	101.09	0.5

RSD : Relative Standard Deviation (five determinations)
*** By AES ** By AAS**
[(a)]Ranbaxy Laboratories, India.
[(b)]Asia Company for pharmaceutical Industries, Sorya.
[(c)]Sun pharmaceutical Industries Ltd.
[(d)]Eli Lilly Company, USA.
[(e)]Bayer Company, Leverkusen, Germany.

was determined in the of 66.4-332 and (1.25-50, 1.25-60) μg.mL^{-1}, respectively.

For Td the reported methods[36,41,42] can not be used due to inadequate sensitivity or interference by endogenous components in plasma. Although the reported methods[43,44] offer high-throughput analysis, they are not readily accessible because of the use of relative expensive tandem mass spectrometry.

In case of Vd the reporte methods[45,50] by Zhu et al. and our previous work in which Vd was determined in the ranges 2.25 – 225 and 1.36-68.32 μg.mL^{-1}, 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. It is clear from the results obtained for the pharmaceutical preparations (TABLE 3) that these excipiences do not interfere.

In order to establish whether the proposed method exhibits any fixed or proportional bias, a siTdle linear regression[56] of observed drug concentration against the theoretical values (five points) was calculated. The student's *t-test*[56] (at 95% confidence level) was applied to the slope of the regression line (TABLE 4), 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 4 : Linear regression analysis for sildenafil, tadalafil and vardenafil using iodobismuthate(III) and iodomercurate(II).

Parameters	$[BiI_4]^{-2}$			$[HgI_4]^{-2}$		
	Sd	Td	Vd	Sd	Td	Vd
Optimum concentration range (μg mL^{-1})	0.948-56.88	0.998-59.88	0.976-58.56	0.948-56.88	0.998-59.88	0.976-58.56
Shift or intercept of the regression line [a]	0.026	0.031	0.029	0.028	0.033	0.032
Slope of regression line	0.9988	0.9978	1.0039	0.9997	1.0029	1.0024
Student's / (2.310)[b]	2.11	2.14	2.10	2.16	2.15	2.09
Range of error (%)	99.89±1.1	100.0±1.2	100.3±1.3	99.87±1.2	99.98±1.1	99.95±1.4

[a]Observed vs theoretical. [b]Tabulated 95% confidence limit (for slope).

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