DRUG FORMULATIONS AND CLINICAL METHODS

Colorimetric Determination of Sildenafil Citrate (Viagra) Through Ion-Associate Complex Formation

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A simple, quick, accurate, and sensitive colorimetric method is described for the determination of sildenafil citrate (SLD). The method is based on the reaction of SLD with Congo Red, Sudan II, and Gentian Violet in buffered aqueous solutions at pH 2.5, 6.5, and 11.0, respectively, to give highly colored soluble ion-associate complex species; the colored products are quantitated colorimetrically at 523, 554, and 569 nm, respectively. The various experimental conditions were optimized. The stoichiometric ratio was found to be 1:1 for all ion associates; the calculated logarithmic stability constants were 8.51, 7.79, and 5.58, respectively. Beer's law was obeyed over the concentration range of 0.2–7.0 μg/mL, whereas the Ringbom optimum concentration range was 0.4–6.5 μg/mL. Values for molar absorptivity, Sandell sensitivity, and detection and quantification limits were also calculated. The proposed method was successfully applied to the determination of SLD in Viagra tablets and in serum samples by using the technique of standard additions with mean accuracy values of 100.06 ± 1.14, 99.87 ± 0.70, and 99.86 ± 0.97% for Viagra tablets and 99.88 ± 0.60, 99.90 ± 0.90, and 100.24 ± 0.80% for serum samples, respectively.

Sildenafil citrate (SLD), 1-([3-(6,7-dihydromethyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxy-phenyl]sulfonyl])-4-methylpiperazine, popularly known as Viagra, is a novel oral agent for the treatment of penile erectile dysfunction, which consists of an inability to achieve or maintain a hard, erect penis sufficient for sexual intercourse (1, 2). It is an active inhibitor of the type V-cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase on penile erectile activity and causes cGMP to accumulate in the corpus cavernosum (3–6). A thorough literature search has indicated that the voltammetric behavior of SLD in pharmaceutical preparations was studied by using square wave and adsorptive stripping techniques (7, 8). A flow injection analysis method using UV detection was reported for the determination of SLD in pharmaceutical preparations (9). However, these methods were found to be unsuitable for differentiating related substances from SLD because of a lack of selectivity.

Various analytical methods have used spectrophotometry (10–12), resonance ray light scattering (13), an ion-selective electrode (14), and reversed-phase high-performance liquid chromatography (HPLC; 15–20) to determine SLD in pharmaceutical preparations. However, a more rapid, sensitive, selective, accurate, and precise method is needed for the determination of SLD.

Ion-associate extraction spectrophotometry (21) has received considerable attention for the quantitative estimation of pharmaceutical compounds. The formation of ion-associate complexes between organic dyes and different organic compounds is one of the techniques available for the determination of pharmaceutical compounds (22–28). This paper describes the development of a colorimetric method that can be used in laboratories without modern and expensive instrumentation such as that required for gas chromatography or HPLC. The proposed colorimetric method involves the formation of ion-associate complexes of SLD with Congo Red (CR), Sudan II (SII), and Gentian Violet (GV) as chromogenic reagents. The proposed procedure was applied successfully to the determination of SLD either in pure pharmaceutical preparations or in serum samples, with good accuracy and precision. The results were compared with those reported earlier.

Experimental

Instrumentation

All absorption measurements were made by using a JASCO 530V (Tokyo, Japan; UV-Vis) spectrophotometer with a scanning speed of 400 nm/min and a band width of 0.2 nm and equipped with 10 mm matched quartz cells. An Orion research model 601A/digital ionalyzer pH meter was used to check the pH of a universal buffer solution prepared as previously recommended (29).

Reagents and Materials

All chemicals used were analytical grade, and all solutions were freshly prepared in bidistilled water. CR, SII, and GV (all from Aldrich, Milwaukee, WI) were used to prepare 1×10^{-3} M

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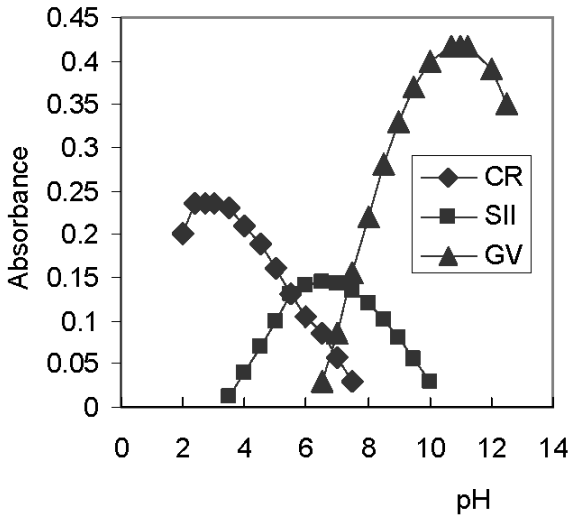

Figure 1. Effect of pH on the absorbance of ion-associate complexes formed by SLD with the chromogenic reagents.

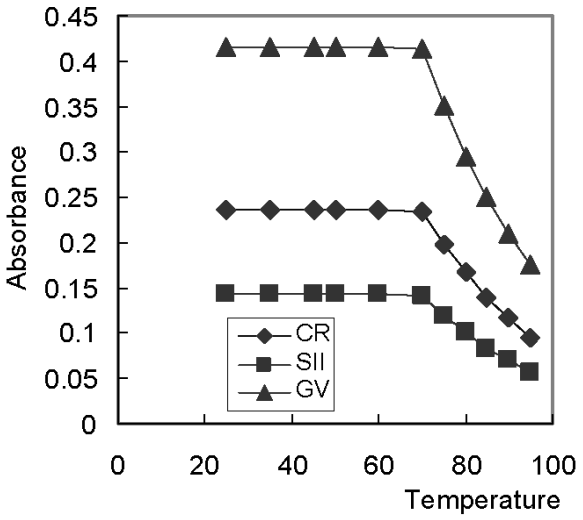

Figure 2. Effect of temperature on the absorbance of ion-associate complexes formed by SLD with the chromogenic reagents.

solutions by dissolving an appropriate weight in 10 mL bidistilled water and diluting to volume with water in a 100 mL volumetric flask. Acetate, borate, phosphate, thiel, and universal buffer solutions with different pH values (2.04–12.56) were prepared as recommended earlier (29).

SLD and Viagra tablets were obtained from Pfizer (New York, NY). A standard stock solution of SLD was prepared by dissolving 50 mg in 15 mL ethanol in a 100 mL volumetric flask and diluting to volume with the same solvent.

General Procedure

In a 10 mL volumetric flask, an aliquot containing SLD at 0.2–70 μg/mL was added to 1.0 mL 10^{-3} M reagent solution (CR, SII, or GV), followed by 5.0 mL universal buffer solution at pH 2.5, 6.5, and 11, respectively. The mixture was diluted to volume with bidistilled water; the solution was allowed to stand for 2.0 min at room temperature (25 ± 1°C), and the absorbance was then measured at λ_{max}, 523, 554, and 569 nm, with CR, SII, and GV, respectively, vs the reagent blank similarly prepared.

Stoichiometric Ratio

By using the molar ratio method, the concentration of SLD is kept constant (0.5 mL of 10^{-3} M), whereas the concentration of the reagent is regularly varied (0.1–1.2 mL of 10^{-3} M). The absorbance of the prepared solutions was measured at the optimum λ_{max} for each ion-associate complex. The values were then plotted vs the molar ratio [reagent]/[drug]. The intersections of the straight lines obtained showed the molar ratio of the most stable complexes.

In the continuous variation method, a series of solutions was prepared by mixing equimolar solutions of SLD and reagent in different proportions, while the total molar concentration was kept constant (1.0 mL of 10^{-3} M). A plot of the absorbance of the solution measured at the recommended wavelength vs the mole fraction of the drug shows a maximum at the molar ratio of the most stable complex.

Procedure for Pharmaceutical Preparations

Five tablets of SLD were weighed and powdered. A quantity of the powdered tablets equivalent to 25 mg SLD was dissolved in ethanol, and the solution was transferred to a 100 mL volumetric flask; the procedure described above was then followed.

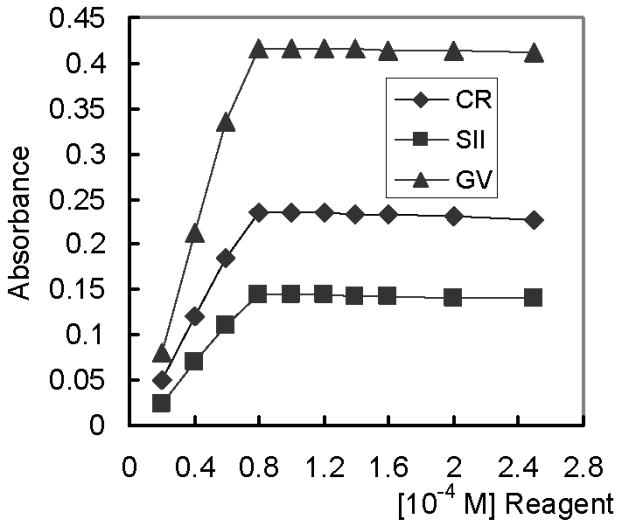

Figure 3. Effect of reagent concentration on the absorbance of ion-associate complexes formed by SLD with the chromogenic reagents.

Procedure for Spiked Plasma

An aliquot of the standard ethanolic solution of SLD at 100 μg/mL was added to 1.0 mL plasma sample in a centrifuge tube; the contents of the tube were mixed for 2.0 min. A 2.0 mL portion of acetonitrile was added for deproteination. The contents of the tube were blended on a Vortex mixer and centrifuged at 2500 rpm for 10 min. The protein-free supernatant was transferred to a small conical flask and evaporated to dryness under a stream of nitrogen at room temperature (25 ± 1°C). The dry residue was dissolved in 10 mL ethanol, and the general procedure described above was followed. A blank value was determined by treating drug-free plasma in the same way. The absolute recovery was determined by comparing the representative absorbance of the plasma sample with the absorbance of the standard SLD at the same concentration.

Results and Discussion

Preliminary investigations revealed that SLD reacts with each of the reagents CR, SII, and GV to yield soluble ion-associate complexes exhibiting absorption maxima at 523, 554, and 569 nm, respectively, whereas the corresponding reagent blanks showed negligible absorbance. Under optimum experimental conditions, the corresponding reagent blanks showed negligible absorbance. Investigations were carried out to establish the most favorable conditions for the ion-associate complexation reaction of the reagents with SLD to obtain maximum color development for the determination. The influence of different variables on the reaction was tested as follows.

Effect of pH

Various aqueous buffers (acetate, borate, phosphate, thiel, and universal buffers) with different pH values were tested to establish the best buffer media. Universal buffer solutions at pH 2.08–12.54 gave the best results; high and constant absorbances were obtained over the pH ranges 2.40–2.70, 6.00–7.00, and 10.7–11.25 by using CR, SII, and GV, respectively. Therefore, all subsequent studies were carried out at pH 2.5, 6.5, and 11.0, because the results were highly reproducible at those pH values (Figure 1). Moreover, the optimum volume of the universal buffer solution was examined and found to be 5.0 mL in a total volume of 10 mL.

Table 1. Analytical characteristics of the proposed procedures

Parameter	CR	SII	GV
pH	2.5	6.5	11.0
λ_{max}, nm	523	544	569
Stability, h	18	24	18
Stability constant, pK	8.51	7.74	5.53
Stoichiometric ratio	1:1	1:1	1:1
Linear concn range, μg/mL	0.2–7.0	0.2–7.0	0.2–7.0
Ringbom optimum range, μg/mL	0.5–6.3	0.4–6.3	0.5–6.5
Detection limit, ng/mL	63	58	60
Quantification limit, ng/mL	195	180	186
Molar absorptivity, $L \cdot mol^{-1} \cdot cm^{-1}$	2.61×10^4	1.62×10^4	4.61×10^4
Sandell sensitivity, $ng \cdot cm^{-2}$	17	27.81	9.61
Regression equation[a]			
Slope	0.059	0.036	0.104
RSD[b] of slope, %	0.016	0.014	0.023
Intercept	–0.003	0.007	–0.008
RSD of intercept, %	0.046	0.039	0.058
Correlation coefficient	0.9986	0.9992	0.9998
RSD, %	1.05	1.22	1.15
Recovery, %	98.9–101.3	98.5–101.10	99.2–100.9
Calculated *t*-value (2.23)[c]	1.74	1.88	1.56
Calculated F_0 value (5.05)[c]	3.34	3.63	2.98

[a] A = a + bC, where C is the concentration in μg/mL.

[b] RSD = Relative standard deviation.

[c] Values in parentheses are the theoretical *t*- and *F*-values at 95% confidence limits and 10 degrees of freedom.

Table 2. Determination of SLD in pharmaceutical formulations (Viagra) by applying the standard additions technique

Reagent	Taken, mg	Added, mg	Found, mg[a]		*t*-Value[c]	*F*-test[c]
			P[b]	HPLC		
CR	0.25	—	0.253	0.255	0.89	1.63
		0.15	0.402	0.406	1.03	2.17
		0.39	0.544	0.555	0.97	1.86
		0.45	0.705	0.690	0.73	1.58
		0.60	0.844	0.838	1.10	2.34
SII	0.15	—	0.148	0.154	0.91	1.72
		0.20	0.352	0.356	1.13	2.50
		0.40	0.553	0.555	1.22	2.88
		0.60	0.745	0.740	1.05	2.26
		0.80	0.942	0.938	1.31	3.14
GV	0.20	—	0.198	0.204	1.17	2.59
		0.25	0.445	0.456	0.93	1.75
		0.50	0.753	0.740	1.00	2.13
		0.75	0.946	0.938	1.42	3.31
		1.00	1.210	1.220	1.15	2.62

[a] Each value is the average of 6 determinations.

[b] P = Proposed method.

[c] Theoretical *t*- and *F*-values at 95% confidence limits and 10 degrees of freedom are 2.23 and 5.05, respectively.

Effect of Time and Temperature

Sample solutions containing SLD and the blank were treated identically with the reagent and buffer for different times and at different temperatures. The results obtained indicated that ion-associate complexes were formed instantaneously at room temperature (25 ± 1°C). The absorption spectra and color intensities were not altered by varying the temperature up to 70°C; above that temperature the absorbance decreased by 15% for every increase of 5.0°C (Figure 2). The absorbance remained stable for ≥18 h, after which it began to decrease slowly.

Effect of Reagent Concentration

When various concentrations of CR, SII, and GV were added to a fixed concentration of SLD at 4.0 μg/mL, 0.8 mL 10^{-3} M reagent solution was found to be enough to develop the color to its full intensity. However, 1.0 mL 10^{-3} M reagent was used in the present study (Figure 3) to ensure a quantitative reaction at the upper limit of the calibration curve.

Stoichiometric Ratio

The stoichiometry of the ion-associate complexes formed between SLD and the reagents CR, SII, and GV was investigated at the optimum pH values by applying the molar ratio and continuous variation methods. The results indicated the formation of a 1:1 ion-associate complex. The logarithmic stability constants were calculated and are shown in Table 1. The presence of the ion-associate complexes was supported by the bathochromic shift observed from 502, 523, and 557 nm for the reagents to 523, 554, and 569 nm, respectively.

Mechanism

The acid dye technique is an ion-associate mechanism in which the ion-associate is formed between the negative ion produced from the ionization of the reagent under consideration, which is converted into $[R]^-$ in the buffer solution, and the positive ion of the drug, $[SLD]^+$. A representative example for the suggested structure of the ion-associate formed between reagent I and any drug can be shown as follows:

$$[GV] + [SLD] \longrightarrow [GV]^-[SLD]^+$$

Method Validation

Under the optimum experimental conditions described, standard calibration curves for SLD with CR, SII, and GV were constructed by plotting absorbance versus concentration. Conformity with Beer's law was evident in the concentration range of the final dilution (Table 1). The linear regression equation for each method is also shown in Table 1. For more accurate results, Ringbom optimum concentration ranges were obtained by plotting transmittance percent versus the logarithmic value of the concentration in μg/mL (Table 1). The correlation coefficients were 0.9986–0.9998, indicating good linearity. Values for mean molar absorptivity, Sandell

Table 3. Assay of SLD in plasma samples

Reagent	Taken, μg/mL	Found, μg/mL[a]	Recovery, %	SD, %[b]	RSD, %[c]
CR	1.4	1.39	99.29	0.69	0.72
	2.8	2.81	100.36	0.43	0.45
	4.2	4.22	100.48	0.52	0.55
	5.6	5.58	99.44	0.48	0.50
	7.0	6.97	99.57	0.57	0.60
SII	1.0	0.99	99.00	0.85	0.89
	2.5	2.52	100.80	0.74	0.77
	4.0	4.03	100.75	0.68	0.71
	5.5	5.46	99.27	0.72	0.75
	7.0	6.95	99.29	0.66	0.69
GV	1.2	1.21	100.83	0.91	0.95
	2.4	2.41	100.42	0.54	0.57
	3.6	3.58	99.44	0.63	0.66
	4.8	4.85	101.04	0.96	1.00
	6.0	6.05	100.83	0.88	0.92

[a] Each value is the average of 6 determinations.
[b] SD = Standard deviation.
[c] RSD = Relative standard deviation.

sensitivity, detection limits, and quantification limits were also calculated and are shown in Table 1.

The reproducibility of the proposed method was determined by analyzing 6 replicate samples, each containing SLD at 4.0 μg/mL in the final assay solution. At this concentration, the relative standard deviation (RSD) values were 0.76, 0.91, and 0.84% for CR, SII, and GV, respectively.

The performance of the proposed method was assessed by calculating *t*- and *F*-values and comparing them with those obtained by HLPC (18). Mean values were obtained by Student's *t*- (for accuracy) and *F*- (for precision) tests at 95% confidence limits and 10 degrees of freedom (30); the results showed that the calculated *t*- and *F*-values did not exceed the theoretical values.

When the results obtained with the proposed method were compared with those obtained earlier (10–12), they showed a better sensitivity and higher accuracy for the nonextractive method, which required less time and had a lower range for microdetermination. The proposed method is highly precise and is simpler and less time consuming than various HPLC methods (15–20). Moreover, the proposed method could be used for the routine determination of SLD in pure form or in pharmaceutical formulations.

The interference of excipients and additives usually present in pharmaceutical formulations and the interference due to the degradation products of SLD were investigated. Preliminary experiments showed that all additives, excipients, and degradation products did not form ion-associate complexes with the reagents studied. These results indicate the high selectivity of the proposed method and the applicability of its use for routine determinations of SLD in pure and dosage forms.

Analytical Applications

The pharmaceutical formulation Viagra (Pfizer) containing SLD was analyzed by the proposed method, and the accuracy of the method was confirmed by comparison of the results with those obtained by HPLC (Table 2). The standard additions method was used, in which variable amounts of pure drug were added to the previously analyzed portion of the pharmaceutical formulation Viagra. Results (Table 2) confirm that the proposed method is not subject to interference by the tablet fillers, excipients, and additives usually used in Viagra formulations (microcrystalline cellulose, anhydrous dibasic calcium phosphate, magnesium stearate, crascarmellose sodium, titanium dioxide, hydroxypropyl methylcellulose, lactose, and triacetin). The proposed method is highly sensitive; therefore, it could be used easily for the routine determination of SLD in its pure form and in its pharmaceutical formulation (Viagra).

Analysis of Spiked Plasma

The high sensitivity attained by the proposed procedure allows the determination of SLD in biological fluids. Peak plasma concentrations of about 3.0 μg/mL of the studied drugs were achieved 1–2 h after administration of a 50 mg dose of each drug by mouth. For plasma samples only, a deproteination process was carried out by using acetonitrile for sample pretreatment; an extraction procedure was not

necessary (31). Table 3 shows the results for the recovery of SLD from spiked plasma samples.

Conclusions

The proposed method is simpler, less time consuming, and more sensitive than the recommended HPLC methods (15–20). The proposed method was advantageous over other reported visible spectrophotometric methods (10–12) with respect to its higher sensitivity that permits the determination of 0.15 μg/mL. No interference from associated excipients, additives, and degradation products was observed. The proposed method can be used for routine analysis and quality control laboratories for the determination of SLD in raw materials and in pharmaceutical formulations, depending on the availability of the chemicals and the nature of other excipients present in the sample.

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