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Extractive Spectrophotometric Determination of Sildenafil Citrate (Viagra) in Pure and Pharmaceutical Formulations

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ABSTRACT

Two simple, rapid and sensitive extractive spectrophotometric methods have been developed for the assay of sildenafil citrate (SC) in pure and pharmaceutical formulations. These methods are based on the formation of chloroform soluble ion-association complexes of SC with brilliant blue G (BBG) and with bromocresol purple (BCP) in KCl-HCl buffer of pH 1.30 (for BBG) and in NaOAc- HCl buffer of pH 3.5 (for BCP) with absorption maximum at 615 nm and 406 nm for BBG and BCP, respectively. Reaction conditions were optimized to obtain the maximum colour intensity. The absorbance was found to increase linearly with increase in concentration of SC, which was corroborated by the calculated correlation coefficient values (0.9991 and 0.9993). The systems obeyed Beer's law in the range of 0.01-4.0 and 0.021-15.0 μg/mL for BBG and BCP, respectively. Various analytical parameters have been evaluated and the results have been validated by statistical data. No interference was observed from common excipients present in pharmaceutical formulations.

Key words: Viagra, extractive spectrophotometric determination, ion-association complex

INTRODUCTION

Sildenafil citrate (SC) is designated chemically as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazole [4,3-d] pyromidine-5-yl)-4-ethoxyphenyl] sulfonyl]-4-methylpiperazine citrate. The physiological mechanism of erection of the penis involves release of nitric oxide (NO) in the *corpus cavernosum* during sexual stimulation. NO then activates the enzyme guanylate cyclase, which results in increased levels of cyclic guanosine monophosphate (cGMP), production of smooth muscle relaxation in the *corpus cavernosum* and inflow of blood. SC has no direct relaxant effect on isolated human *corpus cavernosum*, but enhances the effect of NO by inhibiting phosphodiesterase type 5 (PDES), which is responsible for degradation of cGMP in the *corpus cavernosum*. When sexual stimulation causes local release of NO, inhibition of PDES by SC causes increased levels of cGMP in the *corpus cavernosum*, resulting in smooth muscle relaxation and inflow to the *corpus cavernosum*. SC is official in USP[1]. The reported spectrophotometric methods[2,3] for the assay of SC involve heating at 60 ± 2°C for 10-15 min[2], applicable to higher concentration of the drug[2] (10-260 μg/mL), have less sensitivity[2,3] or less stable (2 hr). This prompted us to develop simple, sensitive and accurate spectrophotometric methods for the determination of SC in pure and pharmaceutical formulations. The proposed methods are more sensitive (ε = 3.28-12.08 × 10^4) and stable (for more than 8 hr) compared to reported methods[2,3]. These methods are based on the formation of chloroform soluble ion-association complexes of SC with BBG and with BCP in KCl-HCl buffer of pH 1.30 for BBG and in NaOAc-HCl buffer of pH 3.5 for BCP.

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MATERIALS AND METHODS

I. *Reagents*

All chemicals used were of analytical or pharmaceutical grade and quartz processed high-purity water was used throughout the experiments. Pharmaceutical grade SC (99.95% pure) was obtained as gift sample from Ajanta Pharma. Ltd., India. A stock solution of SC containing 250 μg/mL was prepared in distilled water. Aqueous solutions of BBG and BCP (each of 0.1%) were prepared separately in high purity water. Series of buffer solutions of KCl-HCl (pH = 1.0-2.2), NaOAc-HCl (pH = 1.99-4.92), NaOAc-AcOH (pH = 3.72-5.57) and potassium hydrogen phthalate-HCl (pH = 2.2-3.6) were prepared by following the standard methods[4-6].

Different dosage forms of SC *viz*., Edegra, Kamaghra, Silighra, Penegra and Caverta were obtained commercially from different firms.

II. *Apparatus*

A Hitachi UV–visible spectrophotometer model U-2001 with 1 cm matched quartz cells was used for the absorbance measurements. The pH measurements were made on a Schott Gerate pH meter CG 804.

III. *Assay Procedure for Pure Drug*

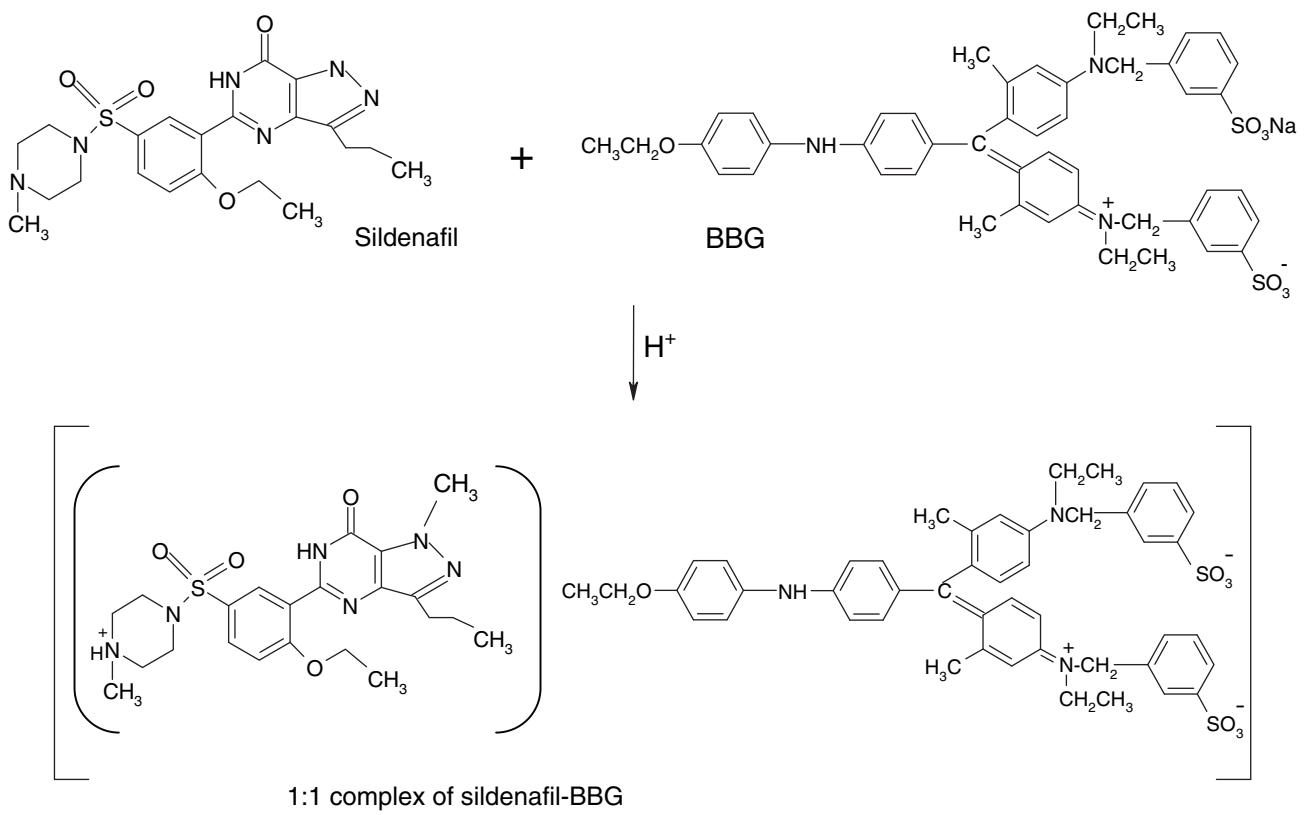
An aliquot of the solution containing 0.1-40 µg (for BBG) or 0.21-150 µg (for BCP) of SC was transferred into a series of 125 mL separating funnels. A volume of 1.5 mL of KCl-HCl buffer of pH 1.30 (for BBG) or 1 mL of NaOAc-HCl (Walpole buffer) of pH 3.5 (for BCP), and 6 mL of BBG or 5 mL BCP were added. Chloroform (10 mL) was added to each separating funnels and then the contents were shaken well and left at room temperature for a minute. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulphate. The absorbances of the blue and yellow coloured complexes were measured at 615 nm and at 406 nm for BBG and BCP, respectively, against the corresponding reagent blank. A calibration graph was plotted.

IV. *Assay Procedure for Tablets*

Six tablets were weighed and powdered. An amount of the powder equivalent to 25 mg of SC was weighed into a 100-mL volumetric flask containing about 70 mL of distilled water. The solution was shaken thoroughly for about 15-20 min, filtered through a Whatman filter paper No. 40 to remove the insoluble matter, and diluted to the mark with distilled water. Twenty five milliliter of the filtrate was diluted to 100 mL and a suitable aliquot was analyzed using the procedure given above.

RESULTS AND DISCUSSION

Since the analyte is a citrate salt of sildenafil, we have considered only sildenafil for further discussion. Sildenafil containing basic functional groups with a pKa value of 8.7 has a weak acidic moiety. In the substituted and fused rings of pyrimidine and pyrazol, protonation is very difficult due to resonance and steric effects. Therefore, the only site in sildenafil vulnerable for protonation is the nitrogen bonded to electron donating methyl group in the piperazine ring. It was observed that the anionic dyes such as BBG and BCP form ion-association complexes with the positively charged drug. The drug-dye stoichiometric ratio as determined by Job's method was found to be 1:1 with BBG and BCP. Each drug-dye complex, with two oppositely charged ions, behaves as a single unit held together by weak electrostatic forces of attraction. Based on these findings, we propose a probable reaction mechanism for the formation of the complexes as shown in Schemes 1 and 2.



Scheme 1. Reaction mechanism of formation of SC-BBG ion pair complex.

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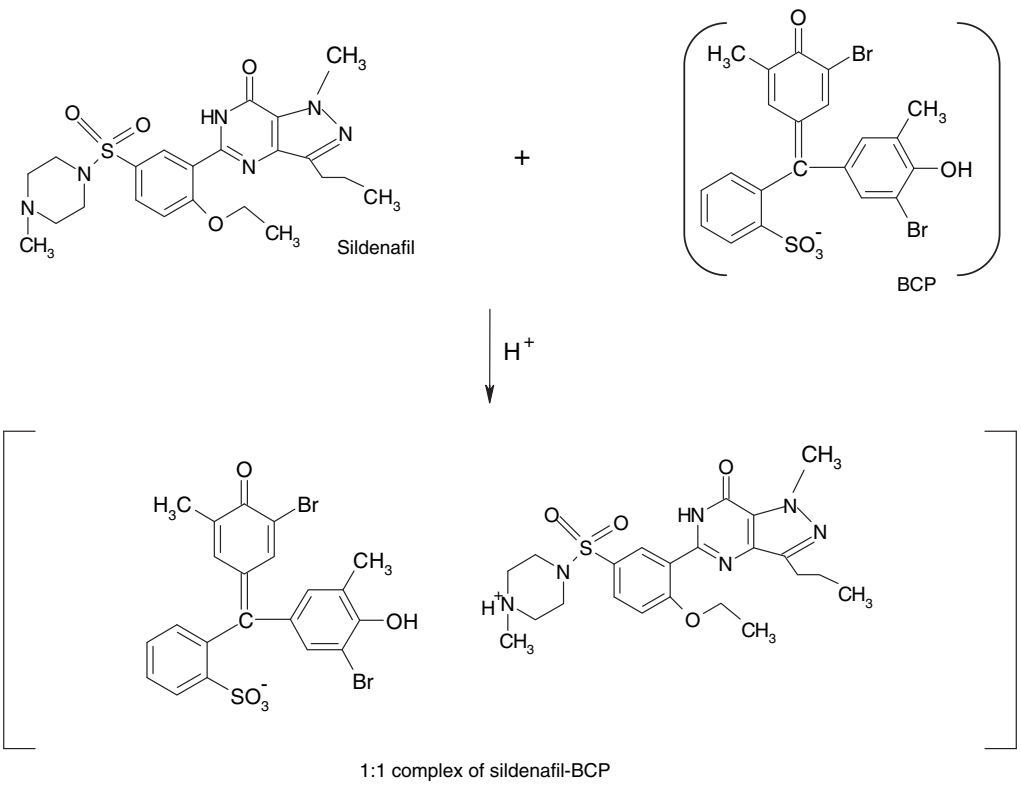
SC reacts with BBG and BCP in acidic buffer to give chloroform soluble ion-association complexes, which exhibit absorption maxima at 615 and 406 nm for BBG and BCP, respectively. Under the experimental conditions, the reagents blank showed negligible absorbance thereby permitting good analytical conditions for quantitative determination of SC.

I. *Optimization of Reaction Conditions*

Optimum reaction conditions for quantitative determination of ion-pair complexes were established via various preliminary experiments. It was observed that the effective extraction of the complex depends on the type of buffer used and its pH. The effect of pH was studied by extracting the coloured complexes in the presence of various buffers such as KCl-HCl (pH = 1.0-2.2), NaOAc-HCl (pH = 1.99-4.92), NaOAc-AcOH (pH = 3.72-5.57) and potassium hydrogen phthalate-HCl (pH = 2.2-3.6). The maximum colour intensity and constant absorbances were observed in KCl-HCl buffer (Clark &Lubs) of pH 1.30 for BBG and in NaOAc-HCl buffer (Walpole) of pH 3.5 for BCP. Further, 1.5 ml of KCl-HCl buffer for BBG and 1 mL of NaOAc-HCl buffer for BCP gave maximum absorbances and reproducible results. Low absorbance values were observed at pH values higher or lower than 1.30 for BBG and higher or lower than 3.5 for BCP in the respective buffer medium. Hence, Clark & Lubs buffer of pH 1.30 for BBG and Walpole buffer of pH 3.5 for BCP were selected for all subsequent measurements. The effects of the reagents were studied by measuring the absorbances of solutions containing a fixed concentration of SC and varied amounts of the reagent separately. Maximum colour intensity of the complex was achieved with 5 mL of 0.1% BBG or with 4 mL of 0.1% BCP Although a larger volume of the reagent had no pronounced effect on the complex formation, the absorbances increased slightly due to background of the coloured reagent. However, 6 mL and 5 mL of BBG and BCP, respectively, were used to ensure complete complex formation. Several organic solvents were tried for effective extraction of the coloured species from aqueous phase. Chloroform was found to be the most suitable extractant since only one extraction was shown adequate to achieve a quantitative recovery of the complex. Shaking times of 0.5 to 2 min produced constant absorbances and hence a shaking time of 1 min was maintained throughout the study. No appreciable change in the absorbance or colour of the product was observed even if the order of addition of the reactants was varied. The experimental data of optimizing reaction conditions are incorporated in Table 1.

II. *Effect of Temperature on the Coloured Complexes*

The effect of temperature on coloured complexes was studied at different temperatures. It was found that the coloured complexes were stable up to 35°C (Table 1). At higher temperatures, the drug concentration was found to increase due to volatile nature of the chloroform.



Scheme 2. Reaction mechanism of formation of SC-BCP ion pair complex.

As a result, the absorbances of the coloured complexes increased. However, the complexes were stable for more than 8 hr at room temperature.

III. *Validation of the Method*

(I) *Detection and Quantification Limits*

According to the Analytical Methods Committee[7], the detection limit (LOD) is the concentration of SC corresponding to a signal equal to the blank mean (Y_B) plus three times the standard deviation of the blank (S_B). Quantification limit (LOQ) is the concentration of SC corresponding to the blank mean plus ten times the standard deviation of the blank.

The LOD values were found to be 4.83 and 7.24 ng/mL for SC with BBG and with BCP, respectively. The LOQ values were observed to be 16.05 and 24.11 ng/mL for SC with BBG and with BCP, respectively. These values indicate that the BBG method is more sensitive than BCP method.

Table 1. Experimental data of optimizing reaction conditions at 3 μg/mL of SC

Buffers	Absorbance	
	BBG	BCG
KCl-HCl	0.62	0.56
NaOAc-HCl	0.53	0.68
NaOAc-AcOH	0.48	0.58
Potassium hydrogen phthalate-HCl	0.45	0.46
pH of buffer	KCl-HCl	NaOAc-HCl
1.0	0.57	0.02
1.2	0.63	0.09
1.3	0.65	0.15
1.5	0.58	0.21
1.6	0.49	0.28
1.8	0.41	0.34
2.0	0.33	0.42
2.2	0.25	0.51
2.5	0.17	0.57
2.7	0.11	0.61
3.0	0.08	0.64
3.2	0.04	0.66
3.5	0.01	0.68
3.7	0.002	0.64
Solvent		
Carbon tetrachloride	0.45	0.44
Ethyl acetate	0.31	0.38
Xylene	0.36	0.41
Chloroform	0.63	0.64
Chlorobenzene	0.28	0.33
Temperature (°C)		
25	0.52	0.55
30	0.58	0.60
35	0.66	0.65
40	0.45	0.41
45	0.37	0.34

(II) *Quantification*

The Beer's law limits, molar absorptivity and Sandell's sensitivity values were evaluated and are shown in Table 2. Regression analyses of Beer's law plots at their respective λ_{max} values revealed a good correlation. Graphs of absorbances *versus* concentration showed zero intercept, and are described by regression equation, Y = bX + c (where Y is the absorbance of a 1 cm layer, b is the slope, c is the intercept and X is the concentration of each selected drug in μg/mL) obtained by the least-squares method. Results are summarized in Table 2.

(III) *Accuracy, Precison and Recovery*

The validity of the methods for the assay of SC was examined by determining precision and accuracy. These were determined by analyzing six replicates of the drug within the Beer's law limits. The low values of relative standard deviation (R.S.D.) indicate good precision

Table 2. Optical characteristics, precision and accuracy data

Parameter	Values	
	BBG	BCP
λ_{max} (nm)	615	406
Beer's law limits (μg/mL)	0.01-4.0	0.021-15
Molar absorptivity (l mol^{-1} cm^{-1})	12.08×10^4	3.28×10^4
Sandell's sensitivity (ng cm^{-2})	5.51	20.32
Stability (h)	8.75	8.50
Correlation coefficient (r)	0.9991	0.9993
Regression equation (Y)[a]		
Slope, b	0.1488	0.0424
Intercept, c	0.0512	0.0452
Relative standard deviation (%)[d]	0.94	0.89
% Range of error[d] (95 % confidence limit)	0.79	0.82
Limit of detection (ng mL^{-1})	4.83	7.24
Limit of quantification (ng mL^{-1})	16.05	24.11

[a]Y = bX + c, where X is the concentration of drug in μg/mL.
[d]Average of six determinations.

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of the methods. The results of analysis of dosage forms are given in Table 3. The results were reproducible as evident from low R.S.D. values.

To study accuracy of the methods, recovery studies were carried out by the standard addition method. For this, known quantities of pure SC were mixed with definite amounts of pre-analyzed formulations such that final concentration of the drug was within Beer's law limits and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference. The results are given in Table 3. The average percent recoveries obtained were quantitative (98.79-99.84 %), indicating good accuracy of the methods.

(IV) *Interference Studies*

The effects of common excipients and additives were tested for their possible interferences in the assay of SC. It was observed that the talc, glucose, starch, lactose,

Table 3. Recovery study for the spiked concentration of SC to the pre-analyzed dosage forms

Formulation	Label claim (mg per tablet)	Amount added (mg)	Amount found* (mg)		Recovery (%)		R.S.D. (%)	
			BBG	BCP	BBG	BCP	BBG	BCP
Edegra	25	5	29.89	30.09	99.56	99.70	0.16	0.13
		10	34.79	34.67	99.40	99.05	0.26	0.42
		15	40.26	39.79	99.35	99.47	0.28	0.24
Kamaghra	50	5	54.76	55.09	99.56	99.84	0.19	0.07
		10	59.83	59.72	99.71	99.53	0.12	0.20
		15	65.19	64.71	99.70	99.55	0.13	0.21
Silighra	100	5	104.59	105.23	99.61	99.78	0.17	0.09
		10	109.34	110.56	99.4	99.49	0.26	0.22
		15	114.37	114.55	99.45	99.61	0.25	0.18
Penegra	50	5	54.51	55.32	99.11	99.42	0.40	0.26
		10	59.62	60.49	99.37	99.18	0.28	0.36
		15	64.21	64.39	98.79	99.06	0.55	0.42
Caverta	50	5	54.69	55.59	99.43	98.93	0.25	0.47
		10	59.61	59.31	99.35	98.85	0.29	0.52
		15	64.66	64.52	99.48	99.26	0.24	0.33

*Average of six determinations.

Table 4. Inter-day precision of the assay of SC by the proposed methods

Drug	Amount taken (µg)	Amount found* (µg)		RSD (%)	
		BBG	BCP	BCP	BCP
Edigra	25	24.89	24.52	0.20	0.87
	50	49.56	48.86	0.39	1.04
	100	99.11	100.41	0.40	0.19
Kamaghra	50	50.33	49.41	0.29	0.53
	100	100.81	98.89	0.36	0.50
Silighra	50	48.96	49.58	0.94	0.39
	100	101.02	100.77	0.45	0.34
Penegra	50	49.01	50.26	0.90	0.23
	100	99.45	98.78	0.25	0.55
Caverta	25	24.51	25.41	0.89	0.72
	50	49.12	50.72	0.80	0.63

*Average of four determinations.

Table 5. Determination of SC in pharmaceutical preparations by the proposed methods and their comparison with reported method[3]

Formulation	Label claim (mg per tablet or capsule	Recovery* ± SD, % and their comparison with reported method		
		Reported method	BBG method	BCP method
Edigra	25	99.28 ± 0.90	99.24 ± 0.75 F = 1.44; t = 1.49	99.31 ± 0.81 F = 1.23; t = 1.57
	50	100.2 ± 0.61	99.14 ± 0.5 F = 1.48; t = 1.55	99.15 ± 0.56 F =1.18; t = 1.67
	100	99.51 ± 0.40	99.32 ± 0.31 F =1.66; t = 1.51	98.94 ± 0.29 F =1.90; t = 1.87
Kamaghra	50	99.41 ± 0.51	99.64 ± 0.44 F =1.34; t = 1.79	99.32 ± 0.39 F =1.71; t = 1.27
	100	100.1± 0.39	100.51 ± 0.32 F =1.48; t = 1.84	99.10 ± 0.28 F =1.94; t = 1.37
Silighra	50	99.18 ± 0.67	101.29 ± 0.6 F = 1.24; t = 1.95	99.56 ± 0.54 F = 1.53; t = 1.71
	100	101.0 ± 0.48	99.71 ± 0.41 F = 1.37; t = 1.86	100.36 ± 0.37 F = 1.68; t = 1.76
Penegra	50	100.7 ± 0.68	99.43 ± 0.65 F = 1.09; t = 1.78	99.71 ± 0.59 F = 1.32; t = 1.84
	100	99.47 ± 0.27	100.76 ± 0.21 F = 1.63; t = 1.85	99.84 ± 0.24 F = 1.26; t = 1.48
Caverta	25	99.04 ± 0.72	99.46 ± 0.66 F = 1.19; t = 1.59	99.67± 0.59 F = 1.48; t = 1.75
	50	102.1 ± 0.62	100.46 ± 0.57 F = 1.18; t = 1.59	99.67± 0.55 F = 1.27; t = 1.75

*Average of six determinations.

sulphate, dextrose, gum acacia and magnesium stearate did not interfere in the determination at the levels normally found in dosage forms.

(V) Ruggedness

To ascertain the ruggedness of the methods, four replicate determinations at different concentration levels of the drugs were carried out. The Intra-day RSD values were less than 1%. The values of Inter-day RSD for different concentrations of drugs obtained from determinations indicate that the proposed method has reasonable ruggedness (Table 4).

IV. *Analysis of Pharmaceutical Formulations, and Statistical Comparison of the Results with Reported Method*[2]

The proposed methods were successfully applied to the analysis of SC in tablets. The results of analysis of pharmaceutical formulations (Table 5) were compared statistically by Student t-test and by the variance ratio F-test with those obtained by reported method. The Student t-values at 95% confidence level did not exceed the theoretical value indicating lack of significant difference between the accuracy of the proposed and reported methods. It was also observed that the variance ratio F-values calculated for $p = 0.05$ did not exceed the theoretical value indicating that there was no significant difference between the precision of the proposed and reported methods.

CONCLUSIONS

Unlike the gas chromatographic and HPLC procedures, the instrument is simple and affordable. The importance lies in the chemical reactions upon which the procedures are based rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility in the assay of a partic-

ular component in complex dosage formulations. The reagents utilized in the proposed methods are cheaper, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The method is unaffected by slight variations in experimental conditions such as pH and reagent concentration. Moreover, the methods are free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control is well established by the assay of SC in pure form and in pharmaceutical preparations.

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