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Utility of Ion-associate Formation Reactions for the Spectrophotometric Determination of Sildenafil Citrate in Pure form and in Virecta Tablets

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Abstract

A simple, rapid and sensitive extractive spectrophotometric method has been developed for the assay of sildenafil citrate (SILC) in pure and pharmaceutical formulations (Virecta tablets). This method is based on the formation of chloroform soluble ion-pair of SILC with bromothymol blue (BTB) and methylene chloride soluble ion-pair of SILC with bromophenol blue (BPB) and eriochrome blue black R (EBBR) in borax buffer of pH 3 and volume 1mL for BTB while acetate buffer of pH 3 and volume 1mL for BPB and universal buffer of pH 2 and volume 1.5 mL for EBBR with absorption maximum at 415, 420 nm and 510 nm for BTB, BPB and EBBR reagents, respectively. Reaction conditions were optimized to obtain the maximum colour intensity. The absorbance was found to increase linearly with the increase in SILC concentration, which was corroborated by the calculated correlation coefficient values (0.9909, 0.9901 and 0.9917 for BTB, BPB and EBBR reagents, respectively). The systems obeyed Beer's law over the concentration range of 1-40, 1-50 and 3-70 μg mL^{-1} for BTB, BPB and EBBR, 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.

Keywords: Virecta tablets; Extractive spectrophotometric determination; Ion-association complex

Introduction

Slidenafil, (5-[2-ethoxy-5-(4-methylpiperazin-1-ylsulfonyl) phenyl]-1-methyl-3 propyl-1, 6-dihydro-7H-pyrazolo [4, 3-d] pyrimidin-7-one), Figure 1 [1], is a potent and competitive inhibitor of the type-V cGMP specific phosphodiesterase enzyme, the predominant isoenzyme in the human corpus cavernosum. Its formula is $C_{28}H_{38}N_6O_{11}S$ and its molecular mass: base: 474.6 g mol^{-1}; citrate: 666.7 g mol^{-1}. Sildenafil enhances relaxation of the corpus cavernosal smooth muscle, which in turn increases blood flow into the cavernosal spaces, thus leading to increased intracavernosal pressure, a key factor in producing an erect penis [2,3]. SILC; sold under the names Viagra and Revatio and under various other names, was a drug used to treat male erectile dysfunction (impotence) and pulmonary arterial hypertension (PAH). However, the introduction of sildenafil resulted to its widespread use as well as its abuse. Therefore, specific, accurate, and robust determination of this drug is widely required. Several methods have been developed for this purpose. Pistos et al. [4] have proposed a HPLC method for determination of sildenafil and its active metabolite (N-desmethyl sildenafil) in human blood. Determination of sildenafil citrate in human plasma [5-8] and in pharmaceutical formulations [9-14] using chromatographic methods have been reported.

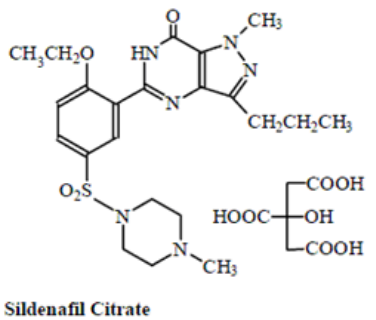

Figure 1: Structural formula of sildenafil citrate.

No official or pharmacopoeial method has been reported for the assay of sildenafil citrate in its formulations. Reports have been appeared describing accurate electroanalytical [15-23] and spectrophotometric [24-32] techniques for quantification and stability assay of SILC. Most of these methods are expensive, suffer from lack of selectivity and require careful control of conditions and considerable time for routine control analysis.

Therefore, precise, sensitive and simple method for the quantification of SILC in pharmaceutical preparations is required. Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs and, therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds. The present communication describes an extractive spectrophotometric procedure for the assay of SILC in pure form and in its formulations, which are based on the formation of ion-pairs with bromothymol blue (BTB), bromophenol blue (BPB) and eriochrome blue black R (EBBR) in acidic buffer.

Experimental

Reagents and materials

All chemicals and reagents used were of analytical reagent grade and some of them were used as such without any further purification. They included sildenafil citrate (SILC) which was provided by EVA

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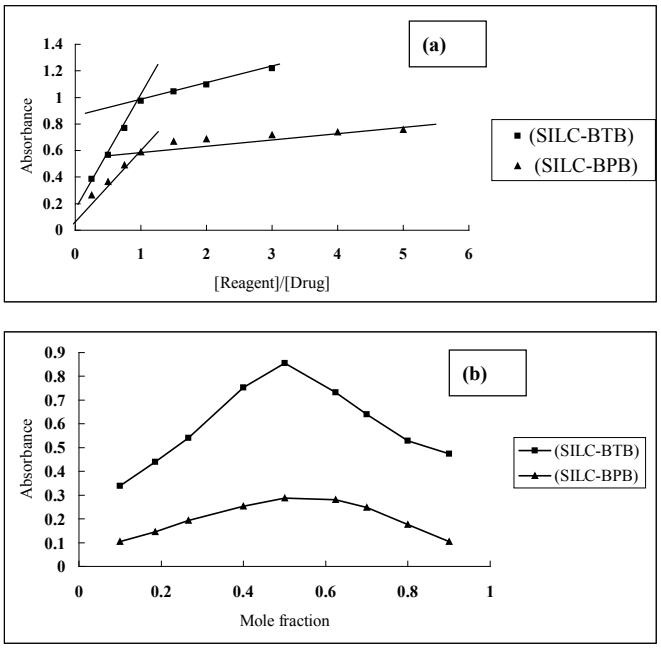


Figure 2: Stoichiometric ratio of the reaction of SILC drug with BTB and BPB reagents using the molar ratio (a) and continuous variation (b) methods.

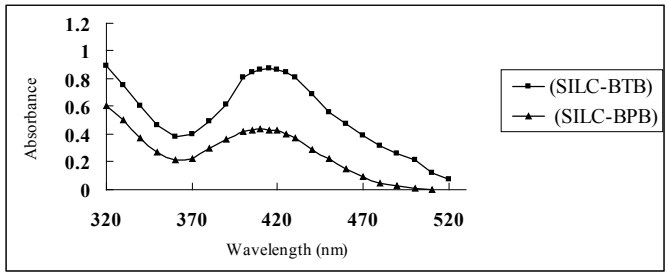


Figure 3: Absorption spectra of SILC ion-pairs using BTB and BPB reagents.

Pharma Company for Pharmaceutical Industry. Dyestuffs included bromothymol blue (BTB), bromophenol blue (BPB) and eriochrome blue black R (EBBR) (were purchased from Win lab, U.K).

Hydrochloric acid was supplied from Merck. While chloroform, methanol, acetone, methylene chloride and ethylene chloride were supplied from El-Nasr Company, Egypt. Potassium chloride, borax, acetic acid, phosphoric acid and boric acid were supplied from El-Nasr Company for Chemicals (Egypt).

Virecta was manufactured by EVA Pharma for Pharmaceuticals and Medical Appliances, Egypt. Each F.C. tablet contains: Sildenafil (as citrate) and labeled to contain 100 mg per tablet.

1 mg mL^{-1} Stock solution of SILC drug was prepared by dissolving the accurate weighed amount in a definite volume of warmed methanol, to get the required concentration. Dilute solutions were prepared by accurate dilution from the stock solution to get the desired concentrations.

0.05% (w/v) solutions of BTB, BPB and EBBR were prepared by dissolving 0.05 g of the reagents in 1: 9 (v/v) methanol: water mixture.

The acid mixture was prepared by mixing 0.04 M solution of each of phosphoric acid (2.597 mL), acetic acid (2.293 mL) and boric acid (2.473 g) in one liter bidistilled water.

Universal buffer solutions of different pH values ranged from 2 to 6 were prepared by adjusting 100 mL solution of the acid mixture to the desired pH value using 0.1 N NaOH solution. Borax and acetate buffer solutions were prepared using the recommended method [33].

Apparatus

Prior to analysis, all glassware used were washed carefully with distilled water and dried in the oven before use.

The spectrophotometric measurements were carried out using the manual Unico 1200 spectrometer (United Products and Instruments, Inc.) in the wavelength range from 190-1000 nm and quarts cell of 1cm optical length was used. Small volumes were taken using automatic pipettes Socorex Swiss (50-200 µL).

Assay procedure for pure drug

In a 10 mL calibrated volumetric flask, 0.5-1 mL of 0.05% w/v BTB, BPB or EBBR solutions were added to 0.1- 0.5 mL of SILC solution (1 mg mL^{-1}), then 1 mL universal buffer (pH = 2), borax buffer (pH = 3) and acetate buffer (pH = 3)) were added in case of BTB, BPB and EBBR, respectively. The volume was completed to the mark with bidistilled water. The mixture had been left for 10, 15-20 and 10 minutes for BTB, BPB and EBBR, respectively, and mixed well in a 50 mL separating funnel then shaked well with chloroform twice (in case of BTB or BPB) or once (in case of EBBR) with 5 mL portions for extracting the ion-pair after shaking well for 1 min. The organic layer was collected in 10 mL measuring flask and the absorption spectra of the resulting solutions were scanned in the wavelength range from 320-600 nm versus chloroform or methylene chloride as blank solution, from which the optimum wavelength for each ion pair was selected.

Assay procedure for virecta tablets

Ten tablets were ground well. A portion of tablets powder equivalent to 100 mg of SILC drug was weighed, then dissolved in the minimum volume of methanol. The solution mixture was shaked in a mechanical shaker and filtered and then transferred accurately to 100 mL measuring flask, completed to the mark with methanol.

Using different concentrations of SILC with BTB, BPB and EBBR reagents which were prepared and the procedure was carried out as mentioned before. The ion-pairs were collected in 10 mL measuring flask. The absorbance of each was measured at its λ_{max} against blank. The drug concentrations were calculated from the standard calibration graph prepared under identical conditions.

Results and Discussion

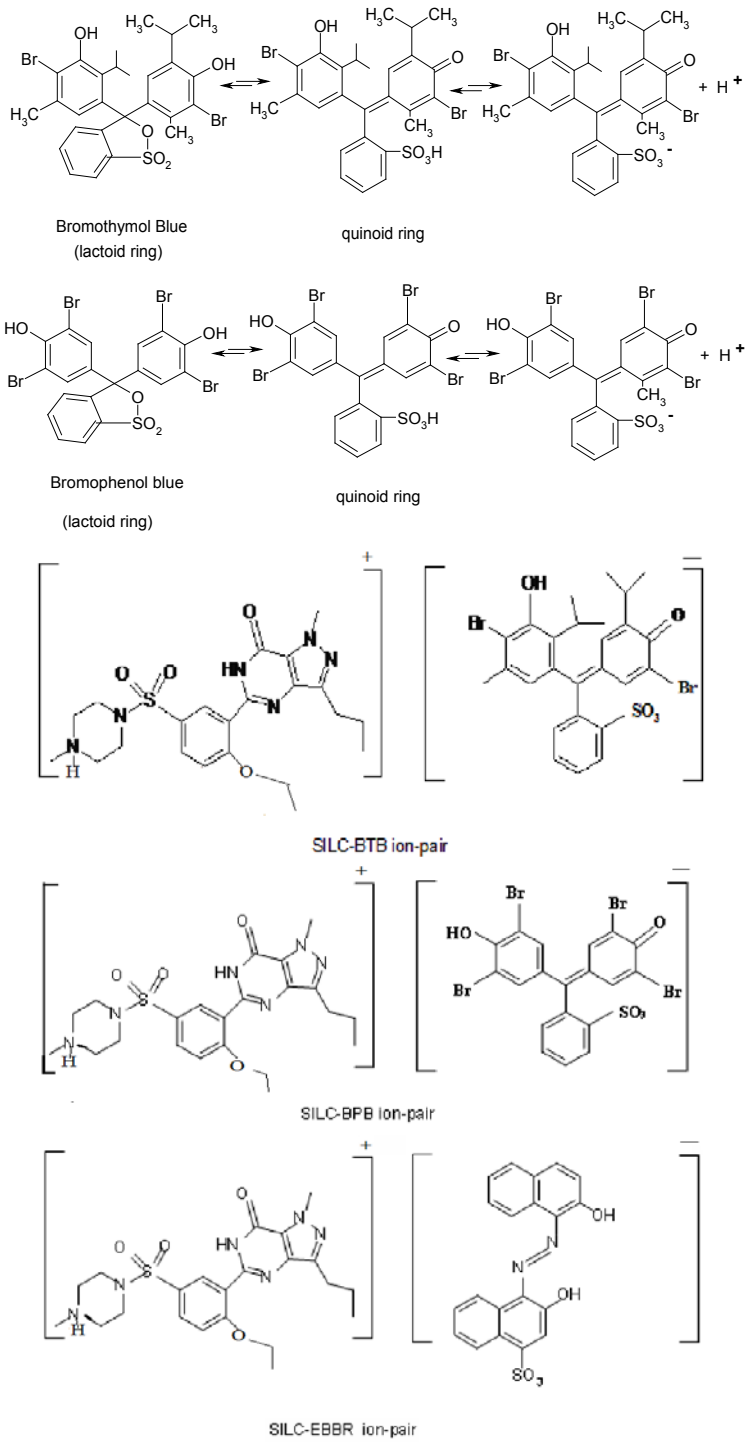
Since the analyte is a citrate salt of sildenafil, sildenafil was considered only for further discussion. Sildenafil containing basic functional groups with a pK_a value of 8.7 has a weak acidic moiety. In the substituted and fused rings of pyrimidine and pyrazol, protonation is very difficult due to 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 [24]. It was observed that the anionic dyestuff reagents namely BTB, BPB and EBBR, form ion-pairs with the positively charged sildenafil drug. The drug-dye stoichiometric ratio was determined by Job's continuous variation [34] and molar ratio [35] methods. It was found to be 1:1 with BTB, BPB and EBBR reagents (Figure 2). Each drug-dye complex, with two oppositely charged ions, behaves as a single unit held together by

weak electrostatic forces of attraction [24,36]. Based on these findings, we propose a probable reaction mechanism for the formation of the ion pairs as shown in Scheme 1.

SILC reacts with BTB, BPB and EBBR reagents in acidic buffer to give chloroform soluble ion-pair with BPB and methylene chloride soluble ion-pairs with BTB and EBBR reagents. The ion-pairs exhibit absorption maxima at 415, 410 and 510 nm for BTB, BPB and EBBR reagents, respectively (Figure 3). Under the experimental conditions, the reagents blank showed negligible absorbance thereby permitting good analytical conditions for quantitative determination of SILC.

Optimization of reaction conditions

Optimum reaction conditions for quantitative determination of ion-pairs were established via various preliminary experiments.



Scheme 1: Structures of SILC-BTB, SILC-BPB and SILC-EBBR ion pairs.

Parameters		BTB	BPB	EBBR
λ_{max} (nm)		415	410	510
time (min)		10	15-20	10
T (°C)		40	Room Temp.	Room Temp.
Conc. Range (µg mL^{-1})		1.00-40.00	1.00-50.00	3.00-70.00
ε (L.mol^{-1}.cm^{-1})		20.12 × 10^4	44.40 × 10^3	24.00 × 10^3
Sandell Senstivity (µg cm^{-2})		0.0033	0.0150	0.0277
A = mC+Z	m	0.0284	0.0221	0.02372
	Z	0.175	0.1406	0.1146
Correlation coefficient (r)		0.9909	0.9901	0.9917
SD		0.015-0.058	0.04-0.062	0.02-0.06
RSD (%)		0.07-0.29	0.20-0.31	0.10-0.30
LOD (µg mL^{-1})		0.85	0.95	0.46
LOQ (µg mL^{-1})		1.28	1.65	1.54
Percentage recovery (%)		100.0–101.0	98.00-101.5	98.50-101.6

Table 1: Analytical parameters for the determination of SILC drug using BTB, BPB and EBBR reagents.

Reagent	[SILC] Taken µg mL^{-1}	[SILC] Found* µg mL^{-1}	% Recovery	SD	RSD (%)
BTB	20.00	20.00	100.0	0.026	0.13
	30.00	30.30	101.0	0.052	0.17
	40.00	40.20	100.5	0.071	0.18
BPB	10.00	9.80	98.00	0.034	0.35
	30.00	30.45	101.50	0.063	0.20
	50.00	49.50	99.00	0.041	0.08
EBBR	20.00	20.00	100.0	0.052	0.26
	40.00	39.40	98.50	0.073	0.19
	60.00	61.00	101.6	0.046	0.08

*Average of four determination

Table 2: Between–day precision for the determination of SILC drug using BTB, BPB and EBBR reagents.

It was observed that the effective extraction of the ion-pair depends on the type of buffer used, volume and its pH. The effect of pH was studied by extracting the coloured complexes in the presence of various buffers of pH = 1-6. The maximum colour intensity and constant absorbances were observed in borax buffer of pH = 3 and volume 1 mL for BTB reagents, while, acetate buffer of pH = 3 and volume 1 mL is optimum condition for BPB reagent and universal buffer of pH = 2 and volume 1.5 mL is optimum for EBBR reagent. The suitable buffer with optimum pH and volume gave maximum absorbances and reproducible results. Low absorbance values were observed at pH values lower than 3, 3 and 2 for BTB, BPB and EBBR reagents, respectively. The effects of the reagents were studied by measuring the absorbances of solutions containing a fixed concentration of SILC and varied concentrations of the reagent separately. Maximum colour intensity of the ion-pairs was achieved with 0.7, 0.2 and 0.4 mg mL^{-1} of BTB, BPB and EBBR reagents, respectively. Although a larger volume of the reagent had no pronounced effect on the ion-pairs formation, the absorbances increased slightly due to background of the coloured reagent. Several organic solvents were tried for effective extraction of the coloured species from aqueous phase. Chloroform was found to be the most suitable extractant with BPB (in accordance to the previously published data [32]) and methylene chloride with BTB (in contrast to the previously published data [32]) and EBBR to achieve a quantitative recovery of the ion-pairs. Shaking times for 1 min was maintained throughout the study.

Effect of temperature and time on the coloured ion-pairs

The effect of temperature on the formed ion-pairs was studied at different temperatures. The results obtained show that, the temperature has no effect on the formation and stability of the ion pairs using BPB and EBBR reagents. The absorbance is generally increased by increasing the temperature and reached a maximum value at 40 °C using BTB reagent. The temperature is slightly increased or decreased above this temperature. Therefore, the room temperature is chosen as the best temperature with BPB and EBBR, and 40 °C with BTB for determination of the drug under study in pure and in pharmaceutical formulations

The effect of time on the formation and stability of the ion-pairs is studied carefully. The absorbance values remain almost unchanged with the increase of time. The optimum time for the completion of the reaction of SILC with BTB, BPB and EBBR reagents is 10, 15-20 and 10 minutes, respectively. The results indicate that ion–pairs need the mentioned time for their complete formation

Validation of the method

Validity of beer's law: Spectrophotometric determination of SILC drug is carried out under the favourable conditions of acidity, suitable buffer, buffer concentration, reagent concentration, time, temperature, ratios, wavelength and extracting solvent.

The results of determination of the drug under investigation are shown in Table (1). The validity of Beer's law for the formed ion-pairs through the reaction of the drug under study with BTB, BPB and EBBR reagents is studied under optimum experimental conditions.

The calibration curves are rectilinear over the concentration range of 1-40, 1-50 and 3-70 µg mL^{-1} SILC drug using BTB, BPB and EBBR reagents, respectively. The mean recovery values obtained amount in the range of 100.0-101.0, 98.00-101.5 and 98.50-101.6% for SILC drug using BTB, BPB and EBBR reagents, respectively. The obtained results indicate the success of the applied procedure in the determination of the studied drug in pure form.

The analytical parameters namely the molar absorptivity (ε), Sandell sensitivity (S), limits of detection (LOD) and quantitation (LOQ), also the regression equation for the drug are summarized in (Table 1).

The low values of the calculated standard deviation and relative standard deviation (SD = 0.015-0.058, 0.04-0.062 and 0.02-0.06, RSD = 0.07-0.29, 0.20-0.31 and 0.10-0.30 % using BTB, BPB and EBBR reagents, respectively), indicate the high accuracy and precision of the proposed method. This is supported also by the calculated values of Sandell sensitivity of 0.0033, 0.0150 and 0.0277 µg cm^{-2} using BTB, BPB and EBBR reagents, respectively. The limits of detection and quantification are calculated and the data obtained are listed in Table (1). The obtained data reflect the sensitivity of dyestuffs reagents (BTB, BPB and EBBR) to the SILC drug determination. The correlation coefficients of the data obtained are found to be 0.9909, 0.9901 and 0.9917 for SILC drug using BTB, BPB and EBBR reagents, respectively.

It is concluded that BTB, BPB and EBBR reagents can be applied successfully for the determination of SILC drug in the concentration ranges mentioned above with high accuracy, precision and sensitivity, as indicated by the values of SD, RSD and Sandell sensitivity.

Between day measurements: The validity and applicability of the proposed dyestuff reagents and the reproducibility of the results obtained can be further proved by carrying out four replicate experiments at three concentrations of SILC drug. Table (2) shows the values of between-day relative standard deviations for different concentrations of the drug obtained from experiments carried out over

Reagent	[Drug] μg mL^{-1} Taken		[Drug] μg mL^{-1} Found		% Recovery		SD	RSD (%)
	Proposed method	SAM*	Proposed method	SAM*	Proposed method	SAM*		
BTB	20.00 40.00		19.90 40.50		99.50 101.3		0.027 0.063	0.14 0.16
BPB	20.00 40.00		20.00 39.00		100.0 97.50		0.018 0.029	0.09 0.07
EBBR	20.00 40.00		20.00 39.50		100.00 98.75		0.036 0.055	0.18 0.14
SAM#	25.00 50.00 75.00	25.00 25.00 25.00		50.57 75.44 100.3		101.1 100.6 100.3	0.046 0.067 0.096	0.09 0.09 0.10

*Amount of standard added, μg mL $^{-1}$
#Percentage recovery using standard addition method

Table 3: Determination of SILC in Virecta tablet using BTB, BPB and EBBR reagents.

Reagent	Solvent	λ_{max} (nm)	Concentration range (μg mL^{-1})	ε L mol^{-1} cm^{-1}	Reference
BCG	Chloroform	415	1.2–25.0	1.58×10^4	[24]
CCR	Chloroform	460	1.5–60.0	9.79×10^3	[24]
chromotrope 2B	Methylene chloride	540	3.3–87.0	1.02×10^4	[25]
chromotrope 2R	Methylene chloride	520	3.3–96.0	8.30×10^3	[25]
3-phenylazo-6-o-carboxyphenylazo-chromotropic acid	Methylene chloride	540	5.0–115.0	6.83×10^3	[25]
bis-3,6-(ohydroxyphenylazo)- chromotropic acid	Methylene chloride	570	2.5–125.0	5.42×10^3	[25]
bis-3,6-(p-N,N-dimethylphenylazo)-chromotropic acid	Methylene chloride	600	8.3–166.7	3.35×10^3	[25]
3-phenylazo-6-o-hydroxyphenylazo-chromotorpic acid	Methylene chloride	575	0.8–15.0	2.32×10^4	[25]
Iodine	1,2-Dichloroethane	366	15–160	3.75×10^3	[28]
TCNQ	Acetonitrile	841	15–220	2.58×10^3	[28]
DDQ	Methanol	460	20–260	2.41×10^3	[28]
TCNE	Acetonitrile	415	10–210	3.05×10^3	[28]
TNF	1,2-Dichloroethane	412	15–240	2.25×10^3	[28]
Chloranilic acid	Acetonitrile	529	20–180	3.26×10^3	[28]
Chloranil	Acetonitrile	550	28–150	3.42×10^3	[28]
Bromanil	Methanol	455	15–170	2.90×10^3	[28]
Congo Red		523	0.2-7.0 mu g/mL		[31]
Sudan 11		554	0.2-7.0 mu g/mL		[31]
Gentian Violet		569	0.2-7.0 mu g/mL		[31]
Methylene blue	Aqueous medium	600	Up to 10.6	3.00×10^4	[40]
Ethyl eosin	Aqueous medium	520	1.3-3.3	2.44×10^4	[41]
BTB	Methylene chloride	415	1- 40	2.01×10^5	Proposed method
BPB	Chloroform	410	1- 50	4.44×10^4	Proposed method
EBBR	Methylene chloride	510	3- 70	2.40×10^4	Proposed method

Table 4: Comparative studies between the proposed and previously reported spectrophotometric methods for determination of SILC.

a period of four days. It is found that, the between day relative standard deviations are less than 3%, which indicates that the proposed method is highly reproducible and successfully applied to determine SILC drug via the formation of ion–pairs with BTB, BPB or EBBR reagents.

Spectrophotometric determination of SILC drug in virecta tablets

The validity of the proposed method is examined for the determination of SILC drug in dosage form manufactured in the local companies. The concentration of the drug in the dosage form is calculated from the appropriate calibration graph. There is no shift in the absorption maximum due to the presence of other constituents of the dosage form.

On screening pharmacopoeia (e.g. USP, BP or EP) it is found that there is no any official method related to determination of SILC in tablet dosage forms or bulk drugs, so we used the standard addition method for testing the proposed methods the values are listed in Table (3) [37-39]. To the preanalyzed formulation, known amounts of the analyte (pure drug) at three different concentration levels were added and assayed. The average percent recoveries obtained were quantitative (98.21–101.8%), indicating good accuracy of the method.

Comparison with the previously reported spectrophotometric methods

Table (4) summarizes the previously reported spectrophotometric [24,25,28,31,40,41] and proposed methods for determination of sildenafil citrate. Comparison with previous literature reported spectrophotometric methods, spectrophotometric determination of SILC applying the proposed methods have advantage over the others. BTB reagent in this study shows higher sensitivity and has higher ε value. Determination of the cited drug through charge transfer reaction with acceptors need long time and heating in water bath to attain the full colour development, but the colour development using the proposed ion-pairs and associates is attained in few minutes (10 min) and at ambient temperature (25°C).

Conclusion

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 particular 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 SILC in pure form and in pharmaceutical preparations.

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