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Spectrophotometric determination of sildenafil citrate drug in tablets. Spectroscopic characterization of the solid charge transfer complexes

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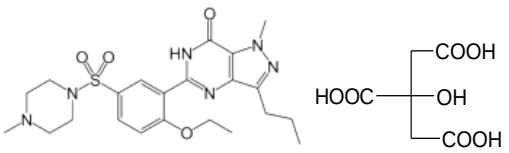
The purpose of this study is to propose sensitive, accurate and reproducible methods for the determination of sildenafil citrate in pure pharmaceutical preparations. Sildenafil citrate is determined spectrophotometrically *via* charge-transfer complex formation. This includes the use of some π-acceptors as 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) and 3,6-dichloro-2,5-dihydroxy-p-benzoquinone (p-CLA). The proposed methods can be used for routine analysis of the suggested drugs in pharmaceutical preparations. The solid ions of the CT complexes from the reaction of DDQ and p-CLA as π-acceptors with sildenafil citrate as donor are isolated and the formed CT complexes are characterized *via* elemental analyses, IR, ^{1}H NMR and mass spectrometric studies.

Keywords: Sildenafil citrate, DDQ, p-CLA, Spectrophotometry, Charge transfer complexes.

1. INTRODUCTION

Sildenafil (S) (1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo-[4,3-d] pyrimidin-5-yl) phenylsulphonyl]-4-methylpiperazine) (Formula 1) has been widely prescribed for treating erectile disfunction [1–4] and its bioavailabilty, metabolism, elimination route and pharmacokinetics have been reported in details [3,5]. The maximum sildenafil plasma concentrations measured after a single oral dose of 100 mg to healthy male volunteers is 450 ng/mL. The lower therapeutic concentrations in human plasma after a 25 mg single oral dose are approximately 7 ng/mL [5]. Many analytical methods, using high performance liquid chromatography (HPLC), have been published for quantification of the parent drug sildenafil in plasma, but not for its active metabolite, using ultraviolet visible (UV–vis) detector [6,7], or a liquid chromatography system combined with a triple quadrupole mass spectrometric detector [8], as well as in oral fluids using a liquid chromatography single mass spectrometry system [9]. Lewis and Johnson [10] reported the detection of both sildenafil and N-desmethylsildenafil in *post-mortem* fluids and tissues, while a liquid chromatography tandem mass spectrometry (LC–MS/MS) system was reported for their detection in urine and tissue samples [11] or in *post-mortem* human blood [12]. Al-Ghazawi *et al*. [13] developed a method for the determination of both analytes in plasma using electrochemical detection; Cooper *et al*. [14] used a UV–vis detector for their determination in plasma; and Saisho *et al*. [15] determined them in human hair by GC–MS. On the other hand, the detection of these compounds without a derivatisation step leads to a higher sensitivity limit. These findings seem to be confirmed not only by the absence of GC–MS methodology for the simultaneous detection of these compounds in the literature, but also by the contradictory results on the parent drug [16, 17].



Formula 1. Structure of sildenafil citrate.

In the present study DDQ and p-CLA reagents are utilized as π-acceptors for the spectrophotometric determination of sildenafil citrate (SILC) drug in raw materials and in some commercial pharmaceutical preparations. Different experimental conditions are checked in order to select the optimum conditions suitable for CT complexes formation and hence quantitative determination of sildenafil citrate (SILC). Statistical treatment of the data obtained, like SD, RSD, Sandell sensitivity, ε, relative error, t- and F-tests are also made.

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2. EXPERIMENTAL

2.1. Materials

All chemicals and reagents were of analytical reagent grade and were used without further purification. Sildenafil citrate (SILC) was provided by EVA Pharma Company for Pharmaceutical Industry. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) was supplied from Arcos-USA. 2,5-Dichloro-3,6-dihydroxy-1,4-benzoquinone (p–CLA) was supplied from BDH chemicals, UK. Absolute ethanol and sodium hydroxide were supplied from ADWIC. Acetonitrile (AR) was supplied from Fisher chemicals and methanol was supplied from Sigma. Chloroform, acetone, 1,4–dioxane, methylene chloride, 1,2-dichloroethane and dimethyl formamide were supplied from El-Nasr Company.

The SILC pharmaceutical preparations were purchased from Silden capsules, 25 mg/cap. (EIPICO) and Virecta, 100 mg/cap. (EVA pharma).

2.2. Solutions

2.1×10^{-3} M SILC solutions were prepared by dissolving accurately weighed amounts of the drugs in warm methanol. 0.1 % (w/v) of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and 0.1 % (w/v) of 2,3-dichloro-5,6-dihydroxy-1,4-benzoquinone (p-CLA) reagents were prepared by dissolving 100 mg of each reagent in 100 ml acetonitrile. All solutions had to be protected from light by keeping them in dark colored quickfit bottles during the whole work. 0.1 M NaOH solution was prepared by dissolving 400 mg of NaOH in 100 ml methanol. Redistilled water from an all-glass equipment was used. Redistillation was carried out from alkaline permanganate solution.

Ten tablets of SILC were accurately weighed and the average weight of one tablet was calculated. The tablets were crushed to a fine powder. A portion of the powder equivalent to 100 mg SILC was dissolved in 75 ml methanol, and then filtered on a dry filter paper in a 100 ml volumetric flask. The volume was brought up to the mark with methanol.

2.3. EQUIPMENTS

All absorption spectral measurements were made using the Perkin Elmer automated spectrophotometer ranged from 200-900 nm with scanning speed 400 nm/min and band width 2.0 nm, equipped with 1 cm matched quartz cells.

Elemental analyses (C, H, N) were made at the Microanalytical center of Cairo University using CHNS-932 (LECO) Vario Elemental analyzers. Infra Red measurements (KBr discs) of the isolated CT complexes were carried out on a Perkin Elmer 1430 ratio recording infrared spectrometer (400–4000 cm^{-1}). ^1H NMR spectra in d_6-DMSO (200 MHz) were recorded on a Varian spectrophotometer Gemini 200 using solvent signals as a reference. ^1H NMR data are expressed in parts per million (ppm). The mass spectra of the CT complexes were recorded at 70 eV by using EI-MS 30 mass spectrometer.

2.4. General procedure for spectrophotometric determination of SOLC

1 ml of 0.1% (w/v) DDQ or p-CLA was added to solutions containing different amounts of 2.1×10^{-3} M SILC. The mixtures were brought up to 10 ml with acetonitrile**.** The absorbance of the colored CT complexes was measured at the specific wavelengths against reagents blank prepared similarly without drugs.

2.5. Day – by – day measurements:

In order to prove the validity and the applicability of the proposed method and the reproducibility of the results obtained, four replicate experiments at different concentrations of SILC were carried out. Using the above mentioned procedures, the absorbance of the four samples was measured daily for four days and the results were recorded to make statistical calculations.

2.6. General procedure for spectrophotometric determination of SILC in some pharmaceutical preparations:

Different concentrations of SILC drug (10–70 μg ml^{-1}) were added to 1 ml of 0.1 % (w/v) DDQ or p-CLA. The volumes were made up to the mark with acetonitrile in 10 ml calibrated measuring flasks. The absorbance was measured at λ_{max} = 460 and 510 nm for SILC using DDQ and p-CLA reagents, respectively, against reagents blank.

2.7. Synthesis of the charge transfer complexes

The solid CT complexes of SILC with DDQ and p-CLA were prepared by mixing a saturated solution of the drug in chloroform (10 ml) and a saturated solution of DDQ or p-CLA in methanol (10 ml) with continuous stirring for about 1 h at room temperature. The colored complexes were developed and the solution was allowed to evaporate slowly at room temperature. The colored

solid complexes formed were filtered, washed several times with little amounts of methanol, and dried under vacuum over anhydrous calcium chloride.

3. RESULTS AND DISCUSSION

Charge transfer complex is the name that is given to a stable molecular system formed in solution between an electron donating molecule, having sufficiently low ionization potential, and an electron accepting molecule having high electron affinity. The main feature of this type of complex formation is the appearance of new intense absorption bands in the ultra-violet or visible region of the spectrum. Absorption bands of this type are known as charge transfer bands, since they involve electronic transitions from an orbital on the donor to a vacant orbital on the acceptor. Many explanations were given to the phenomenon based on the quantum mechanical theory of Mülliken. The formation of molecular complexes from two aromatic molecules could arise from the transfer of an electron from a π–molecular orbital of the donor (Lewis base) to a vacant π–molecular orbital of the acceptor (Lewis acid), i.e. π-π^* electronic interaction [18,19].

3.1. Absorption spectra

The absorption spectra of the SILC-DDQ CT complex in acetonitrile solvent (Figure 1) show three maxima at λ = 460 nm ($\varepsilon^1 = 2.50\times10^3$ L.mol^{-1} cm^{-1}), 510 nm ($\varepsilon^2 = 1.86\times10^3$ L.mol^{-1} cm^{-1}) and 550 nm ($\varepsilon^3 = 2.13\times10^3$ L.mol^{-1} cm^{-1}) while the absorption spectrum of DDQ shows no absorption peaks in the scanned spectral region. The peak at λ = 460 nm was selected because it gives the highest absorption intensity, as indicated by the ε values. Polar solvents such as acetonitrile and methanol were reported to promote the complete transfer of electron from a donor (D) to the π-acceptor (A), DDQ resulting in complete formation of DDQ radical anion (A^-) as a predominant chromogen.

Figure 1 shows the absorption spectra of the SILC-p-CA CT complex in methanol. A solution of drug and p-CLA in acetonitrile and methanol solvents has an intense pink color with characteristic wavelength absorption bands, frequently with one maximum at λ = 510 nm (ε = 0.83×10^3 L.mol^{-1} cm^{-1}), while the p-CLA solution showed a peak at λ = 440 nm (ε = 0.23×10^3 L.mol^{-1} cm^{-1}).

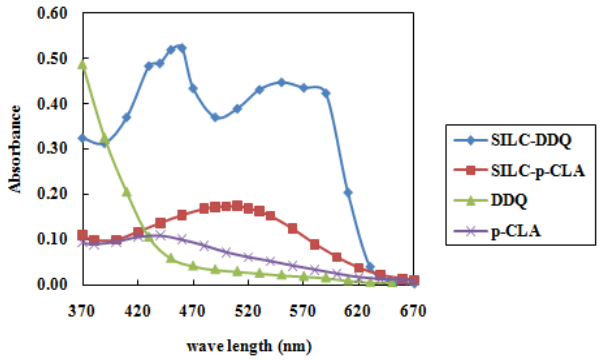


Fig. 1. Absorption spectra of DDQ and p-CLA in acetonitrile and their charge transfer complexes with SILC drug.

3.2. Effect of solvents

In order to select the suitable solvent for CT complex formation, the reaction of DDQ and p-CLA with SILC drug was performed in different solvents. These solvents include acetonitrile, chloroform, ethanol, methanol, acetone, 1,4–dioxane, dichloromethane, 1,2-dichloroethane and dimethyl formamide. The results obtained are shown in Figures (2,3) and Table (1). The DDQ

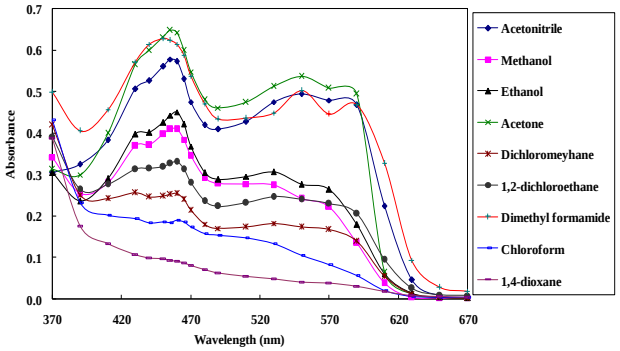


Fig. 2. Effect of organic solvents on the absorption spectra of SILC-DDQ CT complex.

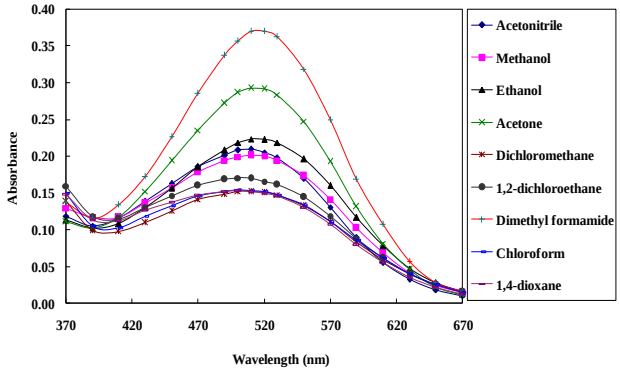


Fig. 3. Effect of organic solvents on the absorption spectra of SILC–p-CLA CT complex.

reagent, acetone and dichloromethane display higher molar absorptivity than acetonitrile. The p-CLA reagent, dimethyl formamide, acetone and ethanol display higher molar absorptivity than

Table 1. The molar absorptivity values of SILC-DDQ and SILC-p-CLA CT complexes in different solvents.

Solvent	Absorbance		ε (L.mol^{-1}. cm^{-1}) * 10^3	
	λ= 460nm	λ= 510nm		
	DDQ	p-CLA	DDQ	p-CLA
Acetonitrile	0.573	0.209	2.73	1.00
Methanol	0.410	0.201	1.95	0.96
Ethanol	0.451	0.223	2.15	1.06
Acetone	0.643	0.292	3.06	1.39
Dichloromethane	0.254	0.151	1.21	0.72
1,2-dichloroethane	0.330	0.269	1.57	1.28
DMF	0.615	0.369	2.93	1.76
Chloroform	0.190	0.153	0.91	0.73
1,4-dioxane	0.090	0.152	0.43	0.72

Table 2. Spectral characteristics of sildenafil citrate CT coloured reaction products and the analytical characteristics (accuracy and precision) of these reactions.

Parameters	Results	
	DDQ method	p-CLA method
λ_{max}, nm	460	510
Molar absorptivity, L. mol^{-1}. cm^{-1}	2.50x10^3	0.83x10^3
Sandell Sensitivity, µg cm^{-2}	0.035	0.043
Beer's law limit, , µg mL^{-1}	10.00 – 100.0	5.00 – 250.0
Percentage recovery, %	98.90 – 100.7	97.80 – 100.1
Range of error, %	0.09 – 1.10	0.01 – 2.20
Standard Deviation (SD)	0.14 – 0.42	0.13 – 0.68
Relative Standard Deviation, (RSD) %	0.20 – 0.84	0.12 – 0.78
Regression equation *, slope (b)	0.0063	0.0016
Intercept (a)	-0.0115	0.0050
Correlation coefficient (r^2)	0.9882	0.9974
LOD, µg mL^{-1}	11.74	12.19
LOD, µg mL^{-1}	9.13	10.63

*A = a + bC; where C is the concentration in µg mL^{-1}.

acetonitrile. But in all cases the best stability and reproducibility of the CT reaction is registered in acetonitrile as a solvent. From these results it follows that acetonitrile can be considered as the ideal solvent for the color reaction, as it offers solvent capacity for DDQ and gives the highest yield of radical anions, as indicated by the high ε values. This is because it possesses the highest dielectric constant among all solvents examined; a property which is known to promote the dissociation of the original CT complex to radical ions, i.e., the dissociation of the donor–acceptor complex is promoted by the high ionizing power of the solvent.

3.3. Effect of reagents concentration

It is found that upon adding various concentrations of DDQ or p-CLA solutions to a constant concentration of ALB, APN and SILC drugs, 1000 µg mL^{-1} of a DDQ or p-CLA solution are sufficient for the quantitative determination of the drug under study, as mentioned above. Maximum and reproducible color intensities are obtained and higher concentration of reagents does not affect the color intensity.

3.4. Effect of time and temperature

The optimum reaction time is determined spectrophotometrically at different time intervals at λ_{max} = 460 and 515 nm for SILC-DDQ and SILC–P-

Table 3. Between – day precision of the determination of SILC drug using DDQ and p-CLA reagents under optimum conditions

Drug	[Drug] Taken, µg mL^{-1}	[Drug]* Found, µg mL^{-1}	Percentage Recovery (%)	SD	RSD (%)
DDQ	20.00	19.59	98.00	0.42	2.16
	30.00	30.58	102.0	0.22	0.72
	50.00	50.54	101.1	0.31	0.61
	90.00	89.86	99.80	0.39	0.44
p-CLA	20.00	19.66	98.31	0.60	2.06
	50.00	50.20	100.4	0.60	1.21
	90.00	90.00	100.0	0.67	0.74
	150.0	149.8	99.87	0.47	0.32

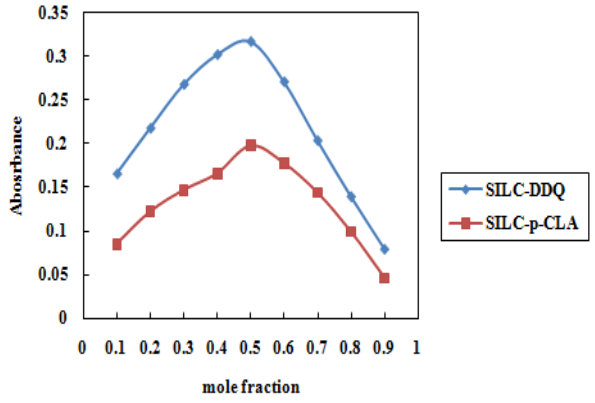


Fig. 4. Job's method for sildenafil citrate CT complexes with DDQ and p-CLA in acetonitrile.

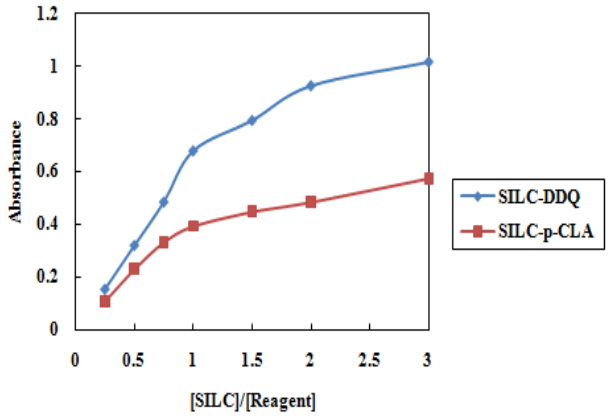


Fig. 5. Molar ratio of sildenafil citrate CT complexes with DDQ and p-CLA in acetonitrile.

CLA CT complexes, respectively. It is found that complete color development is attained after 30 minutes and the color remains stable for one day at least using these reagents.

The effect of temperature in the range from 5 to 60 ºC on DDQ or p-CLA reactions with SILC drug is studied using 100 µg mL^{-1} SILC. It is shown that the maximum color is attained at a temperature of 10±1 or 30±2 ºC for SILC-DDQ and SILC-p-CLA CT complexes, respectively. The color of the CT complexes remained constant for at least 24 h.

3.6. Stoichiometry of the CT complexes

Molar ratio and Job's continuous variation methods [20,21] are applied in order to determine the suitable ratio between SILC drug and DDQ or p-CLA reagents. Figures 4, 5 show that the interaction between these drugs and the reagents occurs on equimolar basis, i.e. the two straight lines are intersected at an 1:1 [Drug]:[Reagent] ratio. This means that 1:1 complexes were formed between the drug and the DDQ or p-CLA reagent. CT complex formation between DDQ or p-CLA and SILC drug takes place through the transfer of electron from a donor (drug) to the π-acceptor reagent (DDQ or p-CLA) [20].

3.7. Validity of Beer's law

Table 2 shows the results of studying the quantitativeness of the reaction between SILC drug and DDQ and p-CLA reagents under the selected optimum conditions. It is found that Beer's law is valid over the concentration ranges from 10 – 100 and 5 - 250 µg mL^{-1}of SILC using DDQ and p-CLA reagents, respectively.

Table 2 shows the slope, intercept, correlation coefficient, Sandell sensitivities, molar absorptivity (ε), range of error, standard deviation, relative standard deviation, limits of detection (LOD) and quantification (LOQ). The low values of Sandell sensitivity indicate the high sensitivity of the proposed method in the determination of the drugs under investigation.

Four to six replicate measurements are performed at different concentrations of ALB, APN and SILC drugs using DDQ and p-CLA reagents. The relative standard deviation and the range of error are calculated. The low values obtained indicate the high accuracy and high precision of the proposed spectrophotometric method. The low values of the limits of detection (LOD) and quantification (LOQ) indicate the possibility of

applying DDQ and p-CLA reagents in routine analysis of the drugs under investigation.

3.8. Between-day precision

In order to prove the validity and applicability of the proposed method and the reproducibility of the results obtained, four replicate experiments at four concentrations of SILC are carried out. Table 3 shows the values of the between-day relative standard deviations for different concentrations of the drugs, obtained from experiments carried out over a period of four days. It is found that within-day relative standard deviations are less than 1%, which indicates that the proposed method is highly reproducible and DDQ and p-CLA reagents are successfully applied to determine SILC *via* the charge transfer reaction.

3.9. Spectrophotometric microdetermination of SILC drug in different pharmaceutical preparations

The spectrophotometric microdetermination of SILC drug in preparations from EPICO and EVA Pharma Companies are carried out. The results obtained are given in Table 4. These data show that the determined concentration of SILC drug by the proposed methods is close to that obtained by the applied standard method [22, 23]. On screening pharmacopoeia (e.g. USP, BP or EP) it is found that there is no official method related to the determination of SILC in tablet dosage forms or bulk drugs, so we used the standard addition method for testing the proposed method.

Table 4. Spectrophotometric determination of SILC drug in different pharmaceutical preparations with DDQ and p-CLA using the standard addition method.

Sample	[Drug] taken, μg mL $^{-1}$	Amount of standard added, μg mL $^{-1}$	[Drug] found, μg mL^{-1}	SD	RSD
Using DDQ: SILC	25.00	--	25.42	0.42	0.89
	50.00	--	49.43	0.31	0.62
	75.00	--	76.43	0.39	0.44
D1	25.00	25.00	50.50	0.71	1.40
	50.00	25.00	75.17	0.43	0.57
	75.00	25.00	100.29	0.50	0.50
D2	25.00	25.00	50.57	0.46	0.91
	50.00	25.00	75.44	0.67	0.89
	75.00	25.00	100.3	0.96	0.95
Using p-CLA: SILC	25.00	--	25.00	0.51	0.31
	50.00	--	50.33	0.60	0.12
	75.00	--	74.67	0.48	0.32
D1	25.00	25.00	50.42	0.88	1.47
	50.00	25.00	75.17	0.51	0.57
	75.00	25.00	99.58	1.00	0.99
D2	25.00	25.00	50.50	1.00	1.75
	50.00	25.00	74.83	0.33	0.89
	75.00	25.00	100.1	0.67	1.00

No. of replicates (n) = 4.
D1 Silden tablets (25 mg/ cap.), EPICO, Cairo, Egypt.
D2 Virecta tablets (100 mg/cap.), EVA Pharma Co., Cairo, Egypt.

Table 4 shows the results obtained by determining the different concentrations of SILC drug using DDQ and p-CLA reagents. It is obvious from these results that the percent recoveries are 98.86 – 101.9 and 99.56 – 100.7 % with DDQ and p-CLA, respectively. These values indicate the accuracy and precision using DDQ and p-CLA reagents.

3.10. Characterization of charge-transfer (CT) complexes

CT complexes formed between SILC drug as donor and DDQ or p-CLA as acceptor were isolated in solid form. The synthesis and characterization of SILC CT-complexes of DDQ and p-CLA were described. These complexes are readily prepared from the reaction of SILC with

DDQ and p-CLA with chloroform and/or methanol solvents. IR, ^{1}H NMR, mass spectra and elemental analyses (C, H, N) were performed to characterize the charge-transfer complexes.

3.10.1. Composition and solubility of the CT-complexes

Results of elemental analysis for the SILC CT complexes are listed in Table (5). From the table it can be seen that the values found are in a good agreement with the calculated ones, and the composition of the CT-complexes matches the stoichiometry (1:1; drug: reagent) of the charge transfer complexes, which is determined by applying continuous variation and molar ratio obtained from a series of solutions of DDQ or p-CLA to SILC. All CT-complexes are insoluble in cold and hot water, but easily soluble in dimethyl formamide and dimethyl sulfoxide.

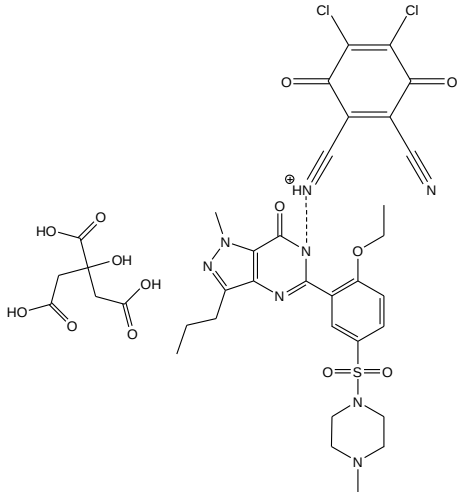
3.10.2. IR spectral studies

The IR spectra of 1:1 CT-complexes formed from the interaction of the donor and the corresponding acceptor with the general formula SILC-acceptor, together with the corresponding free acceptor (DDQ and p-CLA) and SILC donor, are shown in Figure (6 a-e). Full assignments concerning all infrared bands located in the spectra are listed in Table 6.

A comparison of the relevant IR spectral bands of the free donor; SILC, and acceptors; DDQ and p-CLA, with those of their corresponding isolated solid CT-complexes clearly indicates that the characteristic bands of SILC show some shift in the frequencies (Table 6), as well as some change in bands intensities. This could be attributed to the expected symmetry and electronic configuration changes upon the formation of the CT-complex. The explanation will follow separately for each CT-complex to give an idea about the position of complexation.

SILC-DDQ CT-complex

The bands distinguished for the SILC donor in SILC-DDQ CT-complex display small changes in band intensities and frequency values (Figure (6) and Table (6)) . For example, the ν(O-H) vibration occurring at 3473 cm^{-1} for the free donor is shifted to 3439 cm^{-1} with broadening in the IR spectrum of the CT complex. The vibration frequency of the C≡N group for DDQ observed at 2250 cm^{-1} is shifted to 2210 cm^{-1} in the charge transfer complex. The other observation is the blue shift in the δ(NH) deformation from 1581 cm^{-1} in the free donor to 1563 cm^{-1}. From the three mentioned items we can conclude that the charge transfer complexation occurs through interaction between the –NH group of the donor and one of the cyano groups of the DDQ acceptor (Scheme 1).



Scheme 1. Structure of the SILC-DDQ CT-complex.

SILC-p-CLA CT-complex

The infrared spectra of SILC along with its charge-transfer complex SILC-p-CLA are presented in Figure (6), and their band assignments are given in Table (6). The presence of the essential infrared bands of the donor and acceptor in the spectra of the resulting CT-complex, strongly support the formation of a CT complex. Small shifts in both wavenumber values and band intensities between free donor and the CT-complex are registered. This fact is due to the structure configurations upon complexation. For example:

(i) The ν(N-H) vibration of the donor is observed as a medium strong band at 3473 cm^{-1} in the spectrum of the free donor. This band disappears in the spectrum of the complex and so the band due to δ_{def}(N-H) at 1581 cm^{-1} in case of free donor is shifted to 1549 cm^{-1}. This assumption gives an image about the speculative CT complex structure and implies that an –NH group from the donor participated in the complexation.

(ii) The IR spectrum of the SILC-p-CLA CT complex is characterized by two bands (2702 and 2635 cm^{-1}) which are not present in the spectra of the free donor. These bands are assigned to the intermolecular hydrogen bonding [22] between the -NH group (lone pair of electron on nitrogen atom) of the donor SILC with a phenolic OH group in case of p-CLA. Electron-withdrawing groups (chloro groups) attached to the benzene ring increase the acidic property of the phenolic groups present in the p-CLA reagent, accordingly, the phenolic group plays an effective role in the

Table 5. Elemental analysis (C, H, N) and physical parameters of the CT-complexes formed from the reaction of the SILC drug with DDQ and p-CLA reagents.

Complexes(FW)	M wt g/mol		C%		H%		N%		Physical data	
	Found	Calculated	Found	Calculated	Found	Calculated	Found	Calculated	Color	Mp (°C)
SILC-DDQ ($C_{36}H_{38}N_8O_{13}SCl_2$)	929.0	893.7	48.27	48.34	4.23	4.25	12.44	12.53	Yellow	235
SILC-p-CLA ($C_{34}H_{43}N_6O_{15}SCl_2$)	865.0	874.7	46.52	46.65	4.90	4.92	9.44	9.60	Red	225

Table 6. Infrared frequencies[(a)] (cm^{-1}) and tentative assignments for DDQ, p-CLA, SILC-DDQ and SILC-p-CLA CT-complexes.

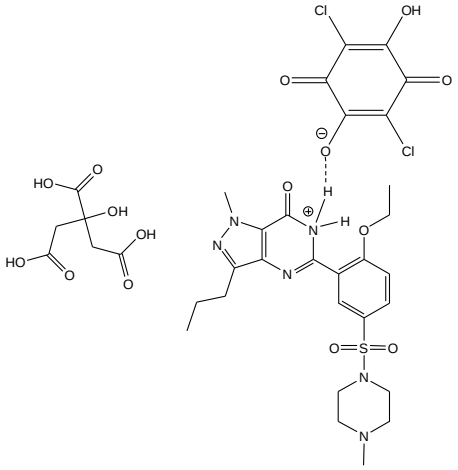
DDQ	p-CLA	SILC	Assignments[(b)]		
			SILC-DDQ	SILC-p-CLA	
3325 w 3218 br	3237 s, br	3615 s 3473 ms 3298 vs 3028 vw	3439 m, br 3305 s 3075 vw 3019 vw	3497 vw 3312 w 3235 ms	$\nu_{(O-H)}$ $\nu_{(N-H)}$ NH_2^+
--	--	2962 w 2869 w 2732 w 2562 w	2970 w	2971 ms	$\nu_{s(C-H)} + \nu_{as(C-H)}$; $-CH_3 + CH_2$
--	--	--	2825 sh 2731 m, br	2702 w 2635 w, br	Hydrogen bonding
2250 vw 2231 ms	--	--	2210 s	--	$\nu_{(C\equiv N)}$
1673 vs	1664 vs	1702 vs	1696 vs 1637 mw	1700 ms	$\nu_{(C=O)}$
--	--	1581 s	1563 vs	1659 w 1631 ms	δ_{def}(N-H) Ring breathing bands
1552 vs 1451 s	1630 vs	1489 w 1461 ms	1460 ms	1549 ms 1458 ms	$\nu_{(C=C)} + \nu_{(C=N)}$+ -COO- C-H deformation+$NH_2^+$ Ring breathing bands
1358 w 1267 s 1172 vs 1072 w	1366 s 1267 s,br	1361 ms 1278 vw 1245 mw 1172 ms 1100 w 1086 w	1394 m 1345 m 1273 mw 1243 w 1193 w 1160 ms 1102 w 1072 vw	1370 ms 1268 s, br 1188 s, br 1162 s, br 1077 sh	$\nu_{(C-C)} + \nu_{(C-N)} + \nu_{(C-O)} + \nu_{(C-S)}$
1010 vw 893 s	980 vs 847 vs	1026 s 937 vs 810 vs	1024 w 938 s 884 m 816 s	1025 sh 979 vs 941 ms 879 vw 837 s	δ_{rock}; NH CH, in-plane bend CH-deformation $\nu_{(C-Cl)}$
800 vs 720 s 615 ms 527 vw 457 ms 432 mw	753 s 688 s 566 s	735 vs 689 ms 658 s 615 s 587 s 560 s 485 s 436 ms	730 ms 688 w 647 w 611 w 580 ms 498 vw 449 vw	750 s 690 s 646 m 615 m 566 vs 494 mw 408 vw	skeletal vibration CH bend CH out-of- plane bend Skeletal vibration $\nu_{(C-S)}$ CNC def. NH_2 rock

(a): s = strong, w = weak, m = medium, sh = shoulder, v = very, br = broad.

(b): ν, stretching; δ, bending

behavior of the acceptors. The proposed mechanism of the interaction between acid and base means that the acidic proton of the acceptor has a tendency to be transferred to the donor (base). This fact can be applied herein in our study of the reaction of p-CLA with SILC as a donor. Such assumption is strongly supported by the existence of new bands of weak intensity (hydrogen bonding) in the spectra of each complex prepared.

(iii) The main stretching vibrations of ν(C=O) and ν(C=C) of the donor are not affected upon complexation, this clearly demonstrating that the lone pair on the nitrogen atom of the –NH group in the pyrimidine ring participates in the complexation process between donor and acceptor. Based on the molar ratio between donor and acceptor which yields 1:1, only one –OH group from the acceptor (p-CLA) is involved in the complexation, as given in Scheme 2.



Scheme 2. Structure of the SILC-p-CLA CT-complex.

3.10.3. ^{1}H NMR spectral studies

^{1}H NMR spectra of ALB, APN, SILC drugs and their six CT- complexes in DMSO are measured and given in Figure (7). The assignments of the spectral data are listed in Table (7). The chemical shifts (ppm) of proton NMR for the defined peaks are determined and listed in Table (7). Evidently, the results obtained from elemental analysis, infrared spectra, and molar ratio titrations met in the same point with ^{1}H NMR spectra to interpret the mode of interaction between donor and acceptor. It is found that the 1H; NH of pyrimidine ring, was up field shifted from 4.11 ppm incase of free donor to 3.83 ppm for SILC-DDQ and 3.99 ppm for SILC-p-CLA, respectively. These suggestions prove the participation of the –NH group of the pyrimidine ring in the CT interaction with one CN group for DDQ and one OH group for p-CLA acceptors *via* intermolecular hydrogen bond.

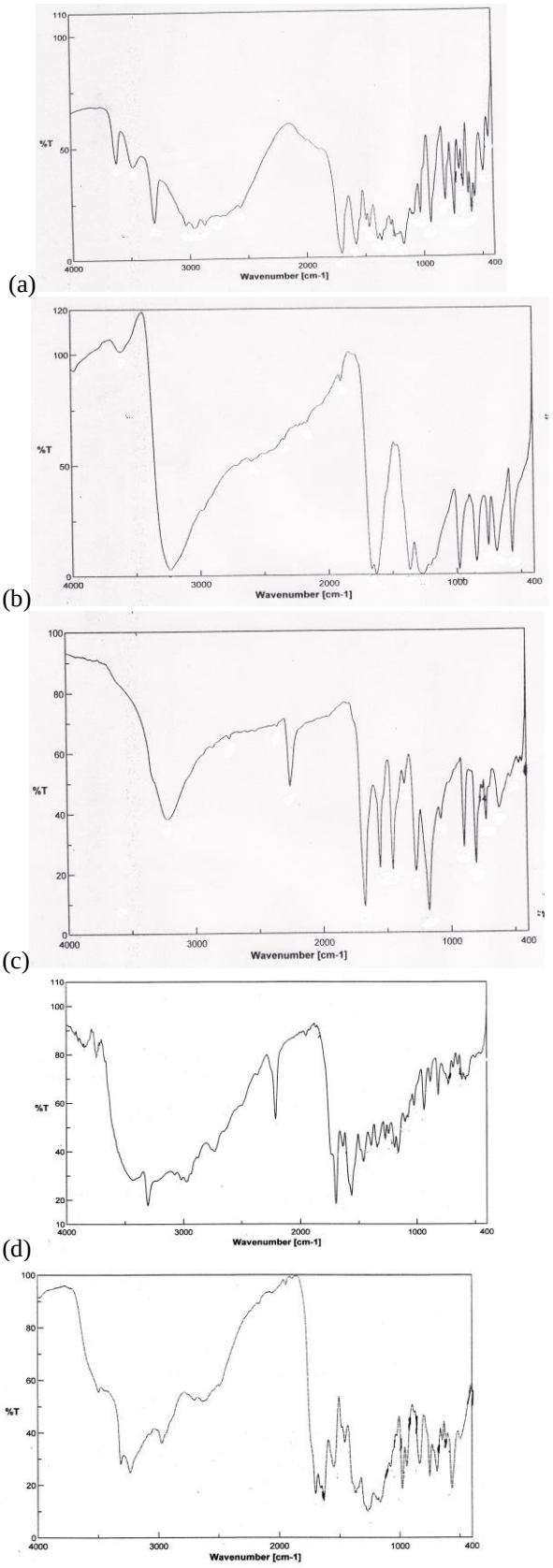


Fig. 6. Infrared spectra of (a) SILC, (b) DDQ, (c) p-CLA,(d) SILC-DDQ, and (e) SILC-p-CLA complexes.

Table 7. ^{1}H NMR spectral data of SILC, DDQ, p-CLA, SILC-DDQ and SILC-p-CLA complexes.

Compound	Chemical shift δ (ppm)	Assignments
SILC	0.873 (m)	3H; CH_3 (Pyrazole-(CH_2)-**C**H_3)
	1.305 (m)	3H; CH_3 (O-CH_2-**C**H_3)
	1.664 (m)	2H; Pyrazole-CH_2-**C**H_2
	2.441-2.616 (m)	2H; Pyrazole-**C**H_2-CH_2 + CH_2 of Piprazine ring
	2.668 (m)	CH_2 and CH_3 of Piprazine ring + CH_2 of citric acid
	2.832 (s)	CH_2 of Piprazine ring + CH_2 of citric acid
	3.065 (s)	CH_2 of Piprazine ring + CH_2 of citric acid
	3.978 (s)	3H; N-**C**H_3 (pyrazole ring) + 2H; CH_3 (O-**C**H_2-CH_3)
	4.105 (s)	1H; NH of pyrimidine ring
	7.337 –7.899 (m)	3H; aromatic ring
DDQ	--	--
p-CLA	8.90 (br)	2H; 2(OH)
SILC-DDQ	0.870 (m)	3H; CH_3 (Pyrazole-(CH_2)-**C**H_3)
	1.341 (m)	3H; CH_3 (O-CH_2-**C**H_3)
	1.734 (m)	2H; Pyrazole-CH_2-**C**H_2
	2.495-2.664 (m)	2H; Pyrazole-**C**H_2-CH_2 + CH_2 of Piprazine ring
	2.678 (m)	CH_2 and CH_3 of Piprazine ring + CH_2 of citric acid
	2.869 (s)	CH_2 of Piprazine ring + CH_2 of citric acid
	3.145 (s)	CH_2 of Piprazine ring + CH_2 of citric acid
	4.123 (s)	3H; N-**C**H_3 (pyrazole ring) + 2H; CH_3 (O-**C**H_2-CH_3)
	3.833 (s)	1H; NH of pyrimidine ring
	7.375 –7.927 (m)	3H; aromatic ring
SILC-p-CLA	0.924 (m)	3H; CH_3 (Pyrazole-(CH_2)-**C**H_3)
	1.341 (m)	3H; CH_3 (O-CH_2-**C**H_3)
	1.690 (m)	2H; Pyrazole-CH_2-**C**H_2
	2.493-2.614 (m)	2H; Pyrazole-**C**H_2-CH_2 + CH_2 of Piprazine ring
	2.666 (m)	CH_2 and CH_3 of Piprazine ring + CH_2 of citric acid
	2.782 (s)	CH_2 of Piprazine ring + CH_2 of citric acid
	3.065 (s)	CH_2 of Piprazine ring + CH_2 of citric acid
	4.126 (s)	3H; N-**C**H_3 (pyrazole ring) + 2H; CH_3 (O-**C**H_2-CH_3)
	3.987 (s)	1H; NH of pyrimidine ring
	7.339 –7.924 (m)	3H; aromatic ring

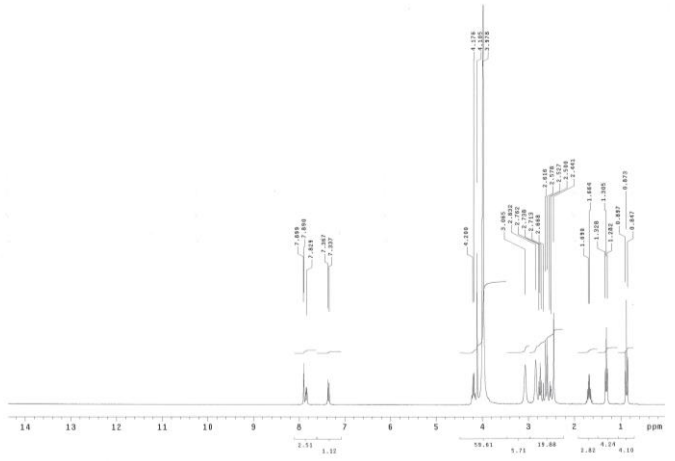
Table 8. Mass fragmentation of SILC, SILC-DDQ and SILC-p-CLA complexes.

Compound	*M/z* (%)[a]
SILC	691(12%), 404(30%), 291(14%), 218(14%), 99(100%), 56(79)
DDQ	227(53%), 200(64%), 165(15%), 137(37%), 110(45%), 87(100%), 52(55%)
p-CLA	209(49%), 188(49%), 145(23%), 123(12%), 105(43%), 87(56%), 69(100%), 52(43%)
SILC-DDQ	865(5%), 827(7%), 765(3%), 730(7%), 663(6%), 625(7%), 506(7%), 409(7%), 376(7%), 341(6%), 304(7%), 271(7%), 227(7%), 193(9%), 157(9%), 120(10%), 58(100%)
SILC-p-CLA	929(12%), 569(15%), 185(20%), 129(12%), 87(100%)

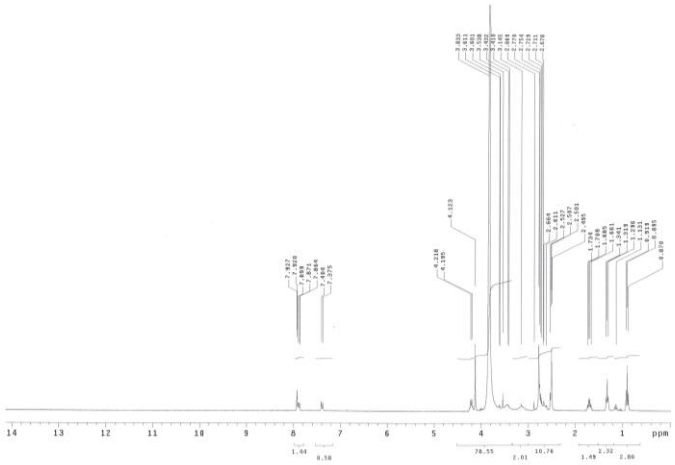
[a] Intensities expressed as % of base peak.

3.10.4. Mass spectral studies

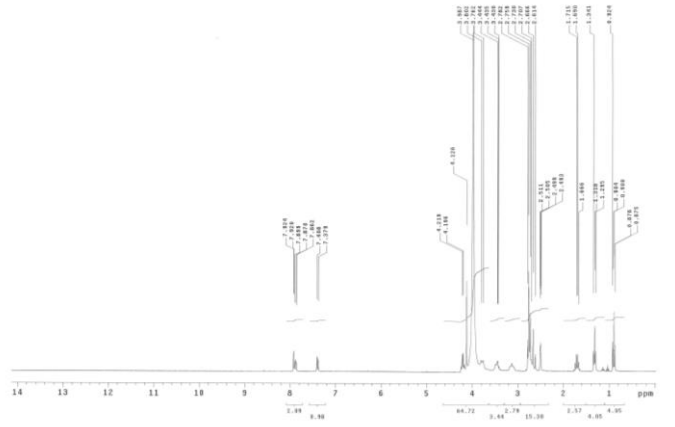
Mass spectrometry was applied to study the purity and the main fragmentation routes of SILC charge-transfer complexes. The differentiation in fragmentation was caused by the nature of the attached acceptors through the intermolecular hydrogen bond between donor/acceptor, while the molecular ion peaks assigned to DDQ m/z = (M+1) 228 (53%), p-CLA m/z = 208(49%), and SILC m/z = 666(12%) are detected in the fragmentation of their CT-complexes. The corresponding mass spectra are given in Table (8). The different competitive fragmentation pathways of the donors give the peaks at different mass numbers listed in Table 8. The intensities of these peaks reflect the stability and abundance of the ions [23].



(a)



(b)



(c)

Fig. 7. ^{1}H NMR spectra of (a) SILC, (b) SILC-DDQ and (c) SILC-p-CLA CT complexes.

4. CONCLUSION

A simple, rapid and reliable spectrophotometric method was adopted for the microdetermination of ABZ drug *via* CT complex formation with DDQ or p-CLA reagents. The effect of different parameters was studied. The results obtained by the suggested procedure were compared with those obtained by the standard method. The data obtained by both procedures were found to be very close to each other and very close to those given by the pharmaceutical companies. The calculated F- and t-tests at the 95% confidence level do not exceed the theoretical values. Also, the formed CT complexes were studied using elemental analyses, IR, ^{1}H NMR and mass spectrometry in order to elucidate the structure of these CT complexes. The results obtained confirmed the results of previous stoichiometric studies and suggested that 1:1 reaction between donors and acceptors takes place; in addition it helped in elucidating the site of interaction between donors and acceptor.

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СПЕКТРОФОТОМЕТРИЧНО ОПРЕДЕЛЯНЕ НА СИЛДЕНАФИЛ ЦИТРАТ В ТАБЛЕТКИ. СПЕКТРОФОТОМЕТРИЧНО ОПРЕДЕЛЯНЕ НА ТВЪРДИ КОМПЛЕКСИ С ПРЕНОС НА ЗАРЯДА

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(Резюме)

Целта на това изследване е да се предложат чувствителни, точни и възпроизводими методи за определянето на силденафил цитрат в чисти фармацевтични препарати. Силденафил цитратът се определя спектрофотометрично чрез образуването на комплекси с пренос на заряда. Това включва използването на някои π-акцепторикато 2,3-дихлоро-5,6-дициано-р-парабензохинон (DDQ) and 3,6-дихлоро-2,5-дихидрокси-р-бензохинон (p-CLA). Предложеният метод може да се използва за рутинен анализ на предлаганото лекарство във фармацевтични препарати. Твърдите йони в комплексите от реакциите с DDQ и p-CLA като π-acceptors и силденафил цитрат като донор са изолирани и образуваните комплекси са охарактеризирани чрез елементен анализ, ИЧ-, ^{1}H ЯМР and мас-спектрометрия.