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Utility of Certain σ and π-Acceptors for the Spectrophotometric Determination of Sildenafil Citrate (Viagra)

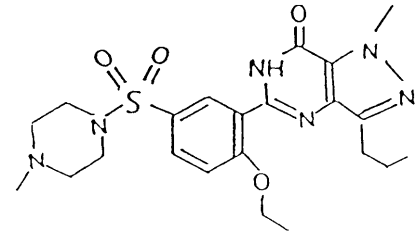
Alaa S. Amin[1,*] and **Ashraf M. El-Beshbeshy**[2]

[1] Chemistry Department, Faculty of Science, Benha University, Benha, Egypt
[2] Quality Control Dep., Egyptian Int. Pharm. Ind. Co. (EIPICO), 10th Ramadan City, Egypt

Abstract. The molecular interaction between sildenafil citrate as electron donor and each of iodine; 7,7,8,8-tetracyanoquinodimethane (TCNQ); 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ); tetracyanoethylene (TCNE); 2,4,7-trinitro-9-fluorenon (TNF); chloranilic acid (CLA); chloranil (CL) and bromanil (BL) as acceptors have been investigated spectrophotometrically. Different variables affecting the reaction were studied and optimized. Beer's law was obeyed in a concentration limit of 10–260 μg/mL for sildenafil citrate. For more accurate analysis, Ringbom optimum concentration range was found to be between 20–240 μg/mL. The limits of detection and determination were calculated and found to be 1.5 and 5.2 μg/mL, respectively. The standard deviations were calculated for different concentrations of sildenafil citrate using various acceptors. A Job's plot of the absorbance versus the molar ratio of the sildenafil citrate to each of acceptors under consideration indicated (1:1) ratio. The proposed methods were found to be rapid, accurate, precise and sensitive and could be applied for determination of sildenafil citrate in pharmaceutical dosage forms (Viagra) without interferences from common additives encountered.

Key words: Sildenafil citrate; charge transfer complexes; spectrophotometry.

Sildenafil citrate is designated chemically as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]-pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methyl-piperazine citrate [CAS 139755-83-2] and has the following structure:



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), producing smooth muscle relaxation in the corpus cavernosum and allowing inflow of blood. Sildenafil has no direct relaxant effect on isolated human corpus cavernosum, but enhances the effect of NO by inhibiting phosphodiesterase type 5 (PDE5), which is responsible for degradation of cGMP in the corpus cavernosum. When sexual stimulation causes local release of NO, inhibition of PDE5 by sildenafil causes increased levels of cGMP in the corpus cavernosum, resulting in smooth muscle relaxation and inflow blood to the corpus cavernosum.

The molecular interactions between electron donors and acceptors are generally associated with the formation of intensely colored charge transfer complexes, which absorb radiation in the visible region [1]. The photometric methods based on these interactions are usually simple and convenient because of the rapid formation of the complexes. Sildenafil citrate is a good

* To whom correspondence should be addressed

n-electron donor and will form charge transfer complexes with σ- or π-acceptors.

σ-Acceptors like iodine and π-acceptors such as TCNE, TCNQ, DDQ, TNF, chloranilic acid (CLA), chloranil (CL) and bromanil (BL) are known to yield charge transfer complexes and radical anions with a variety of electron donors [2–17]. This donor-acceptor interaction has been investigated with sildenafil citrate as electron donor.

This study describes simple, direct, sensitive and precise spectrophotometric methods for the determination of sildenafil citrate via complexation with σ- and π-acceptors in their common dosage forms (Viagra) and irrespective of the presence of contaminants and additives.

Experimental

Apparatus

All absorbance measurements were made with a Shimadzu model 1601 PC and JASCO 530 V spectrophotometer equipped with 10 mm matched silica cells.

Materials

Sildenafil citrate and Viagra tablets were obtained from Pfizer, USA. Standard stock solution of sildenafil citrate was prepared by dissolving 50 mg in 5.0 mL acetonitrile and the volume was diluted to the mark in a 100 mL calibrated flask with acetonitrile.

Reagents

All reagents and solvents used were of analytical-reagent grade. 7,7,8,8-tetracyanoquinodimethane (TCNQ), Aldrich Chem. Co., Milwaukee, USA, and tetracyanoethylene (TCNE), Nacalai Tesque, Kyoto, Japan, 1.0 mg/mL were prepared in acetonitrile, the solutions were stable for at least one week at 4 °C. Chloranilic acid (CLA) and chloranil (CL), Fluka, Switzerland, 2.0 mg/mL in acetonitrile, were prepared fresh daily.

2,3-Dichloro-5,6-dicyano-p-benzoquinone (DDQ), Merck-Schuchardt, Munich, Germany and p-bromanil (BL), Hopkin and William, Essex, England, 2.0 and 5.0 mg/mL in methanol, the solutions were prepared fresh daily.

Iodine resublimed, Riedel De-Haen, Germany and 2,4,7-trinitro-9-fluorenon (TNF), Fluka, Switzerland, 5.0 and 2.0 mg/mL in 1,2-dichloroethane were prepared, the solutions were stable for at least one week at 4 °C.

General Procedure

In 10 mL calibrated flasks, place aliquot volumes containing 100–2600 µg of sildenafil citrate. Add 1.2 mL of reagent and dilute to the mark with the corresponding solvent (Table 1). Measure the absorbance of the solution at the wavelength of maximum charge transfer bands after the appropriate time and temperature against blank treated similarly.

Procedure for Dosage Forms

Five tablets of the drug were weighed and powdered. A quantity of the powdered tablets equivalent to 25 mg drug was dissolved in acetonitrile and transferred into a 50 mL calibrated flask, then the procedure is followed as above.

Molecular Ratio of Reactants in Complex

Job's method of continuous variation was employed, a 2×10^{-3} M standard solution of drug and reagents were used. A series of solutions was prepared in which the total volume of sildenafil citrate and reagent was constant (2.0 mL). The drug and reagents were mixed in various proportions and then diluted in a 10-mL calibrated flask with the optimum solvent. Measure the absorbance after treating each reagent at best time and temperature against a reagent blank under the same conditions.

Association Constant

Serial volumes of 1.0–5.0 mL of 2×10^{-3} M solution (in 1.0 mL steps) in the optimum solvent were transferred in 10 mL calibrated flasks. To each flask, 2 mL of acceptor solution (1×10^{-3} M) was added and continued as described under the general procedure.

Results and Discussion

The immediate change of the violet color of iodine in 1,2-dichloroethane (520 nm) to a lemon yellow color upon reaction with sildenafil citrate was taken as suggestive of charge transfer complex formation which justified scanning in the UV range for the new bands

Table 1. Optimum reaction conditions for sildenafil citrate interaction with various acceptors

Acceptor	Conc, % (mv^{-1})	Solvent	Time, min	Temp, °C	λ_{max}, nm
Iodine	0.06	1,2-dichloroethane	at once	25 ± 1 °C	366
TCNQ	0.12	acetonitrile	10	50 ± 2 °C	841
DDQ	0.24	methanol	5	50 ± 2 °C	460
TCNE	0.12	acetonitrile	5	50 ± 2 °C	415
TNF	0.24	1,2-dichloroethane	15	60 ± 2 °C	412
CLA	0.24	acetonitrile	10	60 ± 2 °C	529
CL	0.24	acetonitrile	10	60 ± 2 °C	550
BL	0.06	methanol	15	60 ± 2 °C	455

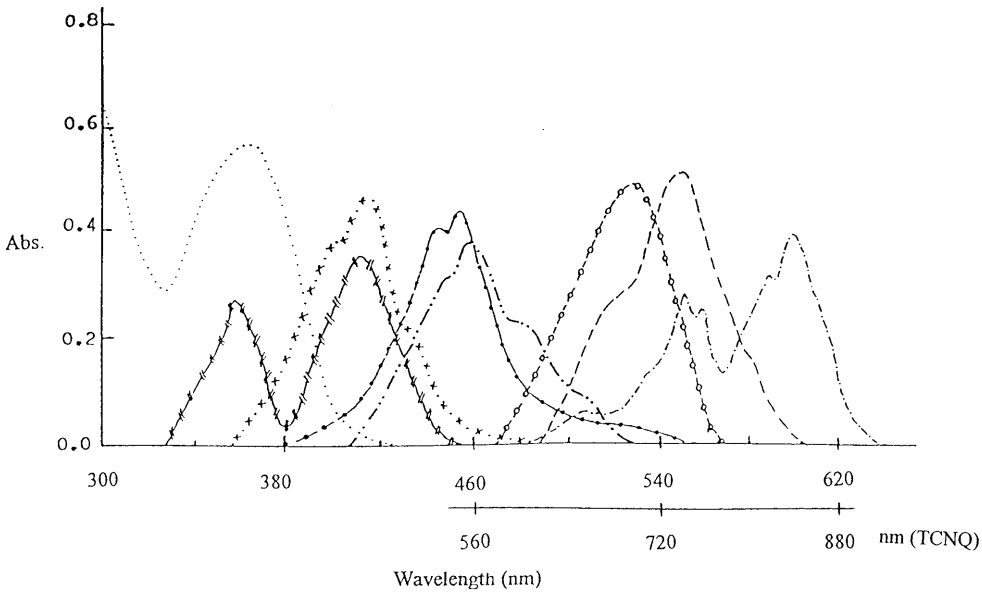


Fig. 1. Absorption spectra of the reaction products of 100 µg ml^{-1} sildenafil citrate with each (....) Iodine, (-.-.-) TCNQ, (-..-..-) DDQ, (x.x.x) TCNE, (#-#-#) TNF, (o-o-o) CLA, (—) CL and (•-•-•) BL

(λ_{max} 366 nm) (Fig. 1). Further confirmation of the charge transfer nature of the reaction was obtained on extracting the drug from the complex by shaking with aqueous mineral acid, whereby the violet color of iodine in 1,2-dichloroethane was restored. All measurements with iodine were performed at 366 nm due to interference from its native UV absorption (Fig. 1).

The interaction of sildenafil citrate with selective polyhaloquinone and polycyanoquinone π-acceptors was found to yield intensely colored radical anions (Fig. 1).

Interaction with TCNQ in acetonitrile solution was found to yield a deep color causing characteristic long wavelength absorption bands. The predominant chromogen with TCNQ is the blue colored radical anion which probably resulted through the dissociation of an original donor-acceptor complex with the drug.

$$D^* + A \rightleftharpoons D^{**} - A \xrightleftharpoons{\text{polar solvent}} D^{*+} + A^{*-}$$

DA complex — radical ions

The dissociation of the complex was promoted by the high ionizing power of acetonitrile solvent [18]. Further support to this assignment was provided by the identity of the absorption maxima with those of the TCNQ radical anion produced by the iodide reduction method in acetonitrile [19].

The resulting maxima of the investigated drug with DDQ, TNF, CLA and CL are similar to that of the radical anion of these acceptors obtained by the reduction method and coincide with the values reported in previous work [20, 21].

For sildenafil citrate and TCNE, the characteristically shaped absorption band of TCNE radical anion with reported maximum in acetonitrile at 432 nm was not formed. Instead a doublet at 394 and 415 nm in acetonitrile was formed which corresponds to the 1,1,2,3,3′-pentacyanopropenide (PCNP) anion. From the quantitative point of view, PCNP anion is preferable to TCNE anion on grounds of its higher molar absorptivity [19]. The small molar absorptivity in case of the reaction between TNF or BL and sildenafil citrate may be due to the insufficient ionization of these relatively weak π-acceptors which possess lower electron affinity than DDQ, TCNQ and TCNE [21].

The results for variation of reagent concentration, indicated that 1.2 mL of either 0.05% iodine and BL, 0.1% TCNQ and TCNE or 0.2% DDQ, TNF, CL and CLA are suitable. The higher concentrations of the reagent may, on the other hand, be useful for rapidly reaching equilibrium and complete color development. This minimizes the time required to attain the maximum absorbance at the corresponding wavelength of the charge transfer complex.

The optimum time was determined by following the color development at ambient temperature ($25 \pm 2\,^{\circ}C$). Complete color development, was attained instantaneously using iodine, whereas for TCNQ, TCNE, DDQ, TNF, CLA, CL and BL complete color development was attained after 60, 40, 40, 75, 60, 50 and 90 min, respectively. To consume the time required for complete color development heating in a water bath at $50 \pm 2\,^{\circ}C$ for 10, 5 and 5 min is sufficient for TCNQ, DDQ and TCNE, respectively, whereas for TNF, CLA, CL and BL, heating at $60 \pm 2\,^{\circ}C$ for 15, 10, 10 and 15 min was the optimal time. The color remained stable for 2.5, 6.0, 4.5, 3.0, 1.25, 3.5, 2.75 and 4.0 h using I_2, TCNQ, DDQ, TCNE, TNF, ClA, CL and BL, respectively. Later the absorbance gradually decreased with a blue shift in λ_{max} until the band disappeared completely.

Choice of Solvent

Although charge transfer complexes are probably formed in many solvents, the high cut-off points of some solvents obscured the scanning of the shorter wavelengths and therefore clear-cut spectroscopic evidence for charge transfer formation could not be ascertained. Also the low solubility of the sildenafil citrate in some other solvents restricted their use.

1,2-Dichloroethane may be used directly as an assay solvent in case of iodine and TNF. With iodine, sildenafil citrate showed a major charge-transfer band at 366 nm.

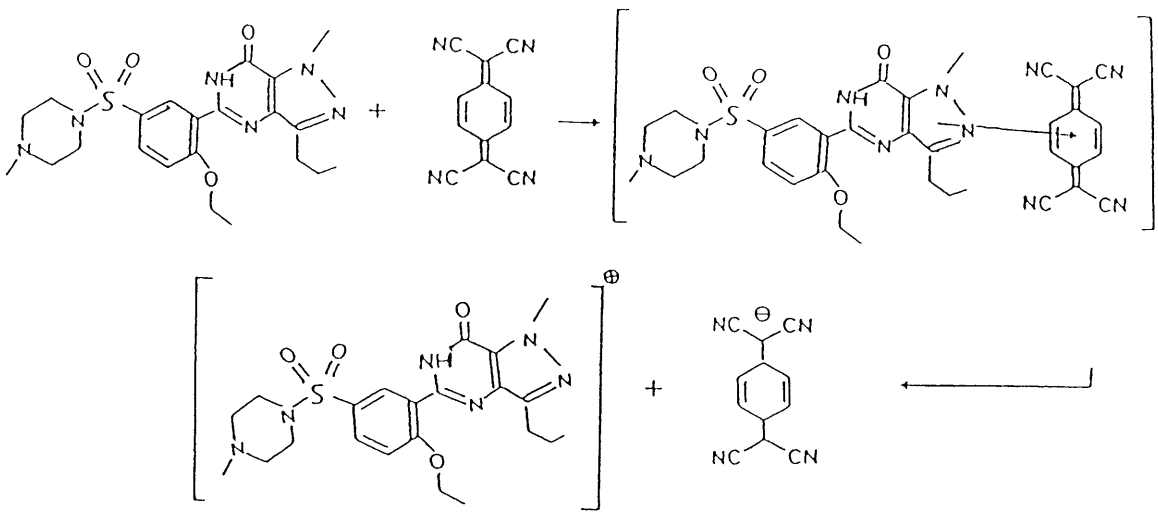
The formation of TCNQ radical was possible in methanol; however, the response between absorbance and concentration was not linear. Furthermore, the molar absorptivity of the obtained chromogen was relatively low compared with that in acetonitrile. The latter was considered as an ideal solvent because it offered excellent solvating power for TCNQ reagent and gave high absorbance.

For TCNE, CLA, CL complex formation, acetonitrile afforded maximum sensitivity when compared with all other solvents (benzene, chloroform, ethylene chloride and methanol). This is because it possesses the highest dielectric constant of all solvents examined [22], a property which is known to promote the dissociation of the original charge transfer complexes to the radical ions. Methylene chloride is a possible candidate, although it suffers from low boiling point which could result in fluctuations of concentration during handling and manipulation. Benzene and chloroform were unsuitable owing to the limited solubility of the reagents [23, 24].

On the other hand, methanol affords maximum sensitivity and full color development with DDQ and BL. In addition, it is a good solvent for the reagent. Of the other solvents examined, dichloromethane and 1,2-dichloroethane are possible substitutes.

Stoichiometric Relationship

Job's continuous variation graph for the reaction between sildenafil citrate and different reagents (Fig. 2) shows that the interaction occurs on an equimolar basis via the formation of a charge transfer complex (1:1). The colored reaction product can be represented, taking TCNQ as an example, by the following structure.



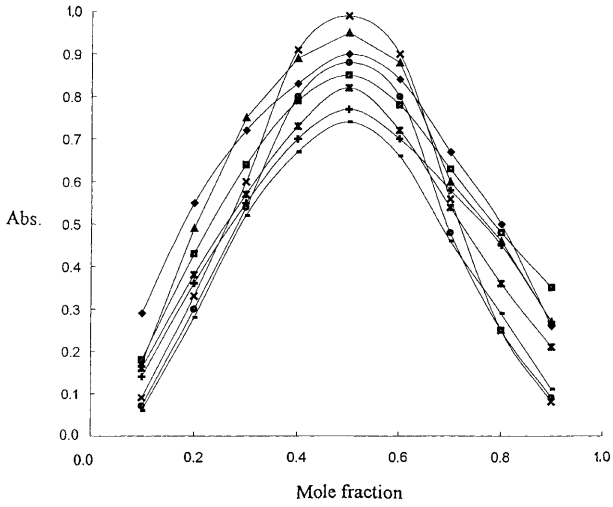


Fig. 2. Continuous variation graph for the reaction of sildenafil citrate with (-◆-) Iodine, (-■-) TCNQ, (-▲-) DDQ, (-x-) TCNE, (-∗-) TNF, (-●-) CL, (-+-) CLA and (—) BL

A more detailed examination was made for sildenafil citrate complexes with the studied acceptors. The absorbance of the complex was used to calculate the association constant using the Benesi-Hildebrand equation [25].

$$\frac{[A_0]}{A_\lambda^{AD}} = \frac{1}{\varepsilon_\lambda^{AD}} + \frac{1}{K_c^{AD}\ \varepsilon_\lambda^{AD}} - \frac{1}{[D_0]}$$

where $[A_0]$ and $[D_0]$ are the total concentration of the interacting species, A_λ^{AD} and ε_λ^{AD} are the absorbance and molar absorptivity of the complexes at their λ_{max}, and K_c^{AD} is the association constant of the complex. On plotting the value of $[A_0]/A_\lambda^{AD}$ vs $1/[D_0]$, a line was obtained with slope equals $(\varepsilon_\lambda^{AD}.K_c^{AD})$ and intercept of this line with the ordinate is $(\varepsilon_\lambda^{AD})$. The calculated association constants are recorded in Table 2, whereas the molar absorptivities are comparable with those obtained from regression line equation of Beer's law.

Quantification

The reproducibility and accuracy of the suggested methods were assessed using different concentrations. The validity was checked occasionally during the work by running six replicate standard samples. Standard

Table 3. Evaluation of precision of the proposed methods

Reagent	Taken $\mu g\,ml^{-1}$	Found* / $\mu g\,ml^{-1}$			
		O	P	S	$S_r\%$
Iodine	30	30.5	29.6	0.03	0.39
	60	60.8	60.5	0.05	0.67
	90	89.1	90.8	0.08	1.05
TCNQ	22	21.8	22.1	0.04	0.55
	44	44.5	43.7	0.09	1.17
	66	66.6	65.6	0.06	0.79
DDQ	35	34.6	35.3	0.10	1.27
	70	69.2	69.5	0.07	0.91
	105	104.0	105.8	0.05	0.69
TCNE	25	24.6	25.2	0.09	1.19
	50	50.9	49.5	0.08	1.07
	75	74.1	75.7	0.11	1.35
TNF	40	40.1	40.3	0.03	0.41
	80	80.7	79.5	0.07	0.95
	120	121.0	119.2	0.09	1.21
CLA	20	19.8	20.1	0.05	0.66
	40	40.4	39.8	0.08	1.03
	60	60.8	59.7	0.10	1.29
CL	23	22.8	23.2	0.06	0.75
	46	46.4	45.5	0.12	1.44
	69	70.7	69.6	0.07	0.93
BL	33	33.5	32.8	0.05	0.67
	66	66.6	66.5	0.12	1.47
	99	98.1	99.8	0.08	1.06

* Average of five determinations. *P* proposed, *O* official method.

Table 2. Quantitative parameters for the complexation of sildenafil citrate with some acceptors

Parameters	I_2	TCNQ	DDQ	TCNE	TNF	CLA	CL	BL
Beer's law limits/$\mu g\,ml^{-1}$	15–160	15–220	20–260	10–210	15–240	20–180	28–150	15–170
Molar absorptivity/$10^3\,L\,mol^{-1}\,cm^{-1}$	3.75	2.58	2.41	3.05	2.25	3.26	3.42	2.90
Sandell sensitivity/$\mu g\,ml^{-1}$	0.178	0.259	0.277	0.219	0.279	0.205	0.195	0.230
Ringbom range/$\mu g\,ml^{-1}$	30–145	25–200	30–254	20–200	25–135	30–165	30–140	25–155
Association constant/log K	3.75	4.1	4.25	3.90	3.60	3.85	3.70	3.85
Detection limits/$\mu g\,ml^{-1}$	1.2	1.2	1.2	1.0	1.2	1.2	1.5	1.2
Determination limits/$\mu g\,ml^{-1}$	5.2	5.3	5.2	5.2	5.3	5.2	5.3	5.3
Regression equation*								
Slope (a)/10^{-3}	5.6	3.9	3.6	4.6	3.4	4.9	5.1	4.4
Intercept (b)	−0.003	+0.007	+0.004	−0.005	−0.007	+0.009	−0.008	+0.006
Correlation coefficient, (r)	0.9996	0.9988	0.9998	0.9992	0.9990	0.9994	0.9996	0.9990
Standard deviation (%)	0.56	0.43	0.38	0.65	0.71	0.87	0.76	0.92
Range of error (%)	±1.1	±0.9	±0.7	±0.8	±1.2	±1.0	±1.4	±1.0

* $A = a + bC$ where C is the concentration in $\mu g\,ml^{-1}$.

calibration curves for sildenafil citrate were prepared by taking series of different concentrations and applying the suggested procedures with iodine, TCNQ, DDQ, TCNE, CLA, CL and BL acceptors. Beer's law is valid within microgram concentration range of sildenafil citrate (Table 1). The regression equations of these calibration graphs were utilized for determination of unknown concentration of sildenafil citrate in tablets. The mean molar absorptivity (ε) and Sandell sensitivity (Ss) as calculated from Beer's law are presented in Table 2. For more accurate analysis, Ringbom optimum concentration ranges were obtained. The standard deviation of the absorbance measurements was obtained from a series of 13 blank solutions. The limits of detection $(k = 3)$ and of determination $(k = 10)$ of the methods were established according to IUPAC definition ($C_1 = kS_0/s$ where C_1 is the limit of detection, S_0 is the standard deviation of blank determination, s is the slope of the standard curve and k is the constant related to the confidence interval [26]) and the values were calculated and recorded in Table 2.

In order to determine the precision of the method, solutions containing six different concentrations of sildenafil citrate were prepared and analysed in quintuplicate. The measured standard deviations (S), (Table 3) can be considered satisfactory, at least for the levels of concentration examined.

Comparison of the recovery obtained with the proposed methods $(100 \pm 1.4\%)$ with the purity of sildenafil citrate as determined according to the

Table 4. Determination of sildenafil citrate in pharmaceutical preparation (Viagra), applying the standard addition technique

Reagent	Sildenafil citrate					
	Taken (mg)	Added (mg)	Found[a] (mg)		t-value[b]	F-test[b]
			O	P		
Iodine	0.30	–	0.305	0.298	0.96	1.69
		0.10	0.407	0.402	1.24	2.00
		0.20	0.495	0.504	1.65	2.48
		0.30	0.590	0.605	1.07	2.04
TCNQ	0.40	–	0.407	0.397	0.89	1.57
		0.75	1.143	1.155	1.31	2.13
		1.50	1.911	1.897	0.81	1.36
		2.25	2.633	2.660	1.44	2.26
DDQ	0.50	–	0.495	0.498	1.00	1.78
		0.50	0.985	1.011	1.36	2.13
		1.00	1.520	1.515	1.71	2.59
		1.50	1.975	2.015	1.53	2.36
TCNE	0.35	–	0.354	0.348	0.79	1.40
		0.15	0.495	0.502	1.15	1.92
		0.30	0.660	0.647	1.48	2.29
		0.45	0.821	0.808	1.21	1.98
TNF	0.25	–	0.254	0.249	0.80	1.42
		0.10	0.354	0.353	0.97	1.73
		0.20	0.440	0.455	1.33	2.07
		0.30	0.535	0.558	1.40	2.21
CLA	0.20	–	0.204	0.199	0.86	1.54
		0.90	1.088	1.111	1.19	1.95
		1.80	1.975	1.990	1.06	1.89
		2.70	2.918	2.888	1.35	2.12
CL	0.15	–	0.147	0.151	0.95	1.64
		0.60	0.766	0.745	1.42	2.17
		1.20	1.375	1.356	1.13	1.88
		1.80	1.940	1.960	1.26	2.04
BL	0.45	–	0.440	0.448	1.01	1.99
		0.40	0.864	0.845	0.87	1.40
		0.80	1.272	1.240	1.34	2.11
		1.20	1.625	1.663	1.58	2.32

[a] Average of six determinations.

[b] Theoretical values for t- and F-tests are 2.57 and 5.05, respectively, for five degrees of freedom and 95% confidence limits.

reference method using HPLC [27] $(99.0 \pm 2.7\%)$ showed a high accuracy of the present methods. The proposed methods are simpler, less time consuming and more sensitive than HPLC method. Moreover, the proposed methods could be used for the routine determination of sildenafil citrate in pure form or in pharmaceutical formulations.

Regarding the interference of the excipients and additives usually presented in pharmaceutical formulation and interference due to the degradation products of sildenafil citrate, the energy of the charge transfer (E_{CT}) depends on the ionization potential (I_P) of the donor and the electron affinity of the acceptor (E_A), hence the λ_{max} values of the other π-donors mostly differ from that of the investigated compounds if they are able to form CT complexes. Preliminary experiments showed that all additives, excipients and degradate products did not form CT complexes with the studied acceptors indicating the high selectivity of the proposed methods and applicability to use for routine determination in pure and in dosage forms.

Analytical Applications

Pharmaceutical preparation Viagra (Pfizer, USA) containing sildenafil citrate was analysed by the proposed methods and the accuracy was assessed by the standard additions method in which variable amounts of pure drug were added to the previously analysed portion of pharmaceutical preparation (Viagra). Results are shown in Table 4 confirming that the proposed methods are not liable to interference by tablet fillers, excipients and additives usually formulated with Viagra (microcrystalline cellulose, anhydrous dibasic calcium phosphate, croscarmellose sodium, magnesium stearate, hydroxypropyl methylcellulose, titanium dioxide, lactose and triacetin). The proposed methods are highly sensitive, therefore it could be used easily for the routine analysis of pure form and in its pharmaceutical preparation (Viagra).

References

[1] R. Faster, *Organic Charge Transfer Complexes*. Academic Press, London. Pp 51 1969, p. 387.
[2] C. N. R. Rao, S. N. Bhat, P. C. Dwivedi, in E. G. Brame (Ed.) *Applied Spectroscopy* Review, Vol. 5. Dekker, New York, 1972, pp. 1–170.
[3] A. S. Amin, G. O. EL-Sayed, Y. M. Issa, *Analyst* **1995**, *120*, 1189.
[4] S. M. AL-Ghannam, A. M. El-Brashy, L. A. AL-Hussein, *J. AOAC Int*. **1999**, *82*, 239.
[5] M. M. Ayad, A. A. Shalaby, H. E. Abdellatef, H. M. Elsaid, *J. Pharm. Biomed. Anal*. **1999**, *18*, 975.
[6] Z. F. Deng, Z. L. Zhu, T. M. Yao, T. H. Li, Liha Jianyan, *Huaxue Fence*. **1999**, *35*, 8.
[7] T. Zheng, S. F. Li, B. Z. Xu, *Yaowu Fenxi Zazhi*. **1999**, *19*, 10.
[8] B. Z. Xu, F. L. Zhao, *Fenxi Ceshi Xuebao*. **1998**, *17*, 29.
[9] F. M. Abdel-Gawad, Y. M. Issa, H. M. Fahmy, H. M. Hussein, *Mikrochim. Acta* **1998**, *130*, 35.
[10] H. E. Abdellatef, *J. Pharm. Biomed. Anal*. **1998**, *17*, 1267.
[11] H. H. Abdine, F. A. EL-Yazbic, S. M. Blaih, R. A. Shaalan, *Spectrosc. Lett*. **1998**, *31*, 969.
[12] F. L. Zhao, B. Z. Xu, S. Y. Tong, *Fenxi Huaxue*. **1998**, *26*, 840.
[13] A. F. M. Albendazole-Wolily, M. A. Korany, A. El-Gindy, M. F. Bedair, *J. Pharm. Biomed. Anal*. **1998**, *17*, 435.
[14] B. K. Rao, Y. S. R. Krishnaiah, S. Satyanarayana, *Indian Drugs* **1998**, *35*, 444.
[15] C. S. P. Sastry, T. V. Rekhe, A. Satyanarayana, *Mikrochim. Acta* **1998**, *128*, 201.
[16] G. A. Salah, *Talanta* **1998**, *45*, 111.
[17] S. R. EL-Shabouri, K. M. Emara, P. Y. Khashaba, A. M. Mohamed, *Anal. Lett*. **1998**, *31*, 1367.
[18] W. Liptay, G. Briegleb, K. Schindler, *Z. Electrochem*. **1962**, *66*, 331.
[19] À. Taha, G. Rücker, *Arch. Pharm. (Weinheim)* **1977**, *310*, 485.
[20] L. R. Melby. In: S. Patai (Ed.) The Chemistry of the Cyano Group, New York, 1970.
[21] A. Yamagishi, *Bull. Chem. Soc. Jpn*. **1975**, *48*, 2440.
[22] The Merck Index, 11 Chem. Edn., Merck, USA, 1990.
[23] T. L. Antonovsky, À. S. Gukovskaja, G. Netrasova, *J. Biochim. Biophys. Acta* **1973**, *331*, 9.
[24] P. M. Qureshi, R. K. Varshey, S. I. M. Kampoonuri, *Spectrochim. Acta* **1989**, *45*, 963.
[25] H. A. Benesi, J. Hildebrand, *J. Am. Chem. Soc*. **1949**, *71*, 2703.
[26] IUPAC Compendium of Analytical Nomenclature, Definitive Rules. In: H. M. N. H. Irving, H. Freiser, T. S. West (Eds.) Pergamon Press, Oxford, 1981.
[27] J. D. Cooper, D. C. Muirhead, J. E. Taylor, P. R. Baker, *J. Chromatogr. B. Biomed. Sci. Appl*. **1997**, *87*, 701.

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