A Simple Spectrophotometric Assay of Sildenafil In Pure and Pharmaceutical Preparations

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Abstract

A simple and rapid method has been established for the determination of sildenafil in pure and pharmaceuticals. The method is based on the formation of ion-association complex of sildenafil citrate (SC) with metacresol purple (MCP) in acidic buffer solution. The complex can be extracted into chloroform and quantitatively measured at 410 nm. Regression analysis of the standard plot reveals that Beer's law was obeyed in the concentration range of 3-70 mg L^{-1} with detection limit of 0.13 mg L^{-1} and sandell sensitivity of 0.0452 μg.cm^{-2}. The accuracy of the method is evaluated on the basis of recovery test which found to be of 100.2 ±0.42%. The proposed procedure can be applied for the quality control of pure and commercial tablets determination of sildenafil citrate.

Keywords: Sildenafil, Metacresol Purple, Tablets, UV-Vis Spectrophotometry.

Introduction

Sildenafil (Viagra) chemically named as 1-[4-ethoxy-3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo [4, 3-*d*] pyrimidin-5-yl) phenylsulfonyl]-4-methylpiperazine citrate [M.wt. 666.6 g/mol], is an anti-impotent drug and a selective inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase type 5 (PED5). The chemical structure of sildenafil is shown in Fig.(1) [1-3].

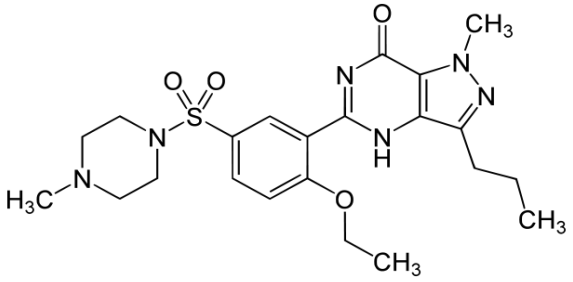


Fig.(1) The chemical structure of Sildenafil.

Drug quality control is a branch of analytical chemistry that has a wide impact on the public health, so the development of a simple, fast and accurate method for the active ingredient determination is welcomed [4]. To date, few reports have been appeared in the chemical literatures such as, spectrophotometry [5-6], LC-ESI-MS [7] HPLC [8], voltammetry [9], and potentiometry [10]. The techniques used for assay of sildenafil such as the aforesaid chromatographic and hyphenated techniques, all require highly sophisticated instruments which are relatively expensive and hence are not always available. Availability of UV-Vis spectrophotometer in many laboratories and the simplicity of analytical procedures make the technique very attractive tool for wide range of applications, including detection of metals and analyses of organic compound such drugs and medicaments.

In the present work, we report that the development of simple, fast, cost-effective and sensitive extractive spectrophotometric assay based on the formation of chloroform soluble ion-pair complex between sildenafil citrate with chrmogenic reagent namely metacresol purple (MCP). All factors that affect the formation of the ion-pair complex were optimized. The validity of the method is evaluated via the analytical characteristics that represented by the figures of merit. The proposed method is suitable for the assay of sildenafil citrate in tablets and promising for the routine quality control of this medicament.

Experimental

Apparatus

A UV-Visible spectrophotometer model UV-1650 PC (Shimadzu, Japan) with 1 cm matched cells was used for all absorbance measurements. The pH values of solutions were measured using GLpH / ORP meter Model HI98150 (HANNA, Romania).

Reagents

All the reagents and chemicals used were of analytical grade. Double distilled water was used to prepare all solutions. M-cresol purple (MCP, Hopkin and Williams LTD), (M.wt. 382.43 g/mol) chroform (BDH), methanol (BDH), KCl and HCl (Fluka) were used without further purification.

Sildenafil citrate (SC) was generously supplied by the Drug Industries and Medical Appliances (SDI), Samarra/Iraq. All commercial SC tablets were purchased from Iraqi drugstores; Vegon (Atlantis Life Sciences, India), Excegra (Excel Life sciences, UK) each tablet was labelled to contain SC of 100 mg, KAM-GRA (Ajanta Pharma Limited, India) contain 50 mg.

Two series of buffer solutions; (a) KCl/ HCl (pH=1-2) was prepared by mixing 50 ml of 0.2M KCl and several mls of 0.2M HCl and (b) NaOAc/AcOH (pH=3-4) was prepared by mixing different proportion of 0.1M sodium acetate (trihydrate) and 0.1M acetic acid [5]. The MCP solution of 1000 mg L^{-1} was prepared by exactly dissolving 0.1 g of MCP in 5 mL methanol and diluted to 100 mL with water.

A stock solution of sildenafil citrate (1000 mg L^{-1}) was prepared by dissolving 100 mg of pure SC in 5 mL of 0.05N HCl and diluted to 100 mL with water. The working standard solutions were then prepared by suitable dilutions of the stock solution with water.

Procedures

Direct Calibration Method

Aliquots (0.15 to 3.5 mL) of 500 mg L^{-1} standard sildenafil citrate corresponding to (3-70 mg SC L^{-1}) transferred into 25 mL volumetric flask. Then, 2.5 mL of 200 mg L^{-1} MCP and 5 mL of buffer solution (pH =1.8) were added and diluted to mark with water. The solutions were set aside for 4 min for complete complex formation. Each solution was extracted with 5 mL of chloroform after shaking for 1.5 min. After the two phases were allowed to separate, the chloroform layer was transferred into a cuvette (1 cm) and the absorbance of the orange coloured complexes was measured at 410 nm against the corresponding reagent blank. The analytical curve was obtained by plotting absorbance against SC concentration and the corresponding linear regression equation was used to convert absorbance into SC concentration, for all analyzed tablets samples.

Preparation of Drug Sildenafil tablets

10-tablets of SC were crushed in a clean agate mortar, powdered and triturated well. A quantity equivalent to one tablet was taken and dissolved in 0.05N HCl and sufficient water with continuous shaking, and then the content was filtered and the filtrate was transferred into 100 mL volumetric flask and diluted to mark with water.

Standard Additions Method

Aliquots (2.5 ml) from above-prepared SC sample solution were pipetted into thirteen 25-mL calibrated flaks containing 0.00, 0.25, 0.50, 0.75. 1.00, 1.25, and 1.50, 1.75, 2.00, 2.25, 2.50, 2.75 and 3.00 mL of 500 mg SC L^{-1} then 2.5 mL of 200 mg L^{-1} MCP and 5 mL of buffer solution (pH =1.8) were added and diluted to mark with water. The solutions were set aside for 4 min for complete complex formation. The extraction process was carried out according to the procedure previously mentioned under direct calibration method.

Mole-Ratio Method

An aliquot (2 ml) of a solution 2×10^{-4} M of sildenafil solution was added to a series of 25 ml volumetric flask containing 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2 and 3.6 ml of 2×10^{-4} of MCP. To each flask 5 ml of buffer solution (pH = 1.8) was added and dilute to the mark with water. The complex formed in each flask was extracted with chloroform and the absorbance of the extract was measured at λ_{max} (410 nm). The absorbance versus the volume ratio of MCP/SC was plotted (Fig.7) from which the stoichiometry of ion-association complex was determined.

Results And Discussion

Absorption Spectra

UV-Vis spectra of the pure SC drug, pure MCP and the complex SC-MCP were scanned using Shimadzu model UV-1650 PC equipped with 1 cm matched quartz cell for recoding the spectra to verify of the formation of complex. It was shown that the pure drug gave two absorption maxima at 225 and 292 nm and the spectrum of the pure MCP shows distinctive absorption maximum at 435 nm, while the

SC-MCP complex gave an absorption maximum at 410 nm (Fig.2) indicating the formation of complex between the drug and chromgenic reagent in $CHCl_3$.

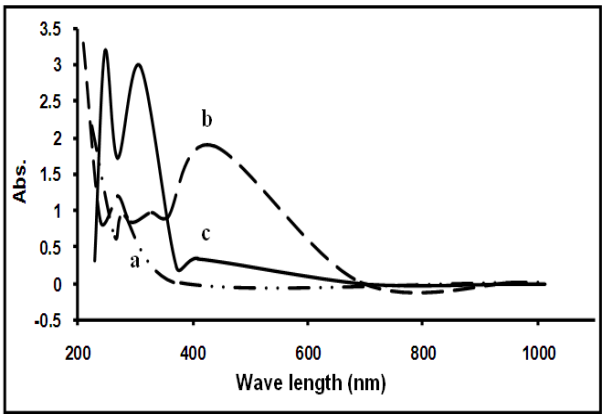


Fig.(2) The molecular absorption spectra of (a) SC (50 mg L^{-1}); blank water, (b) MCP (20 ppm); blank water, and (c) SC-MCP complex (25 mg L^{-1} + 20 ppm + buffer solution) in $CHCl_3$; blank $CHCl_3$.

Optimization of variables

- **Effect of pH value**

The effect of pH on the formation of SC-MCP complex forming from 45 mg L^{-1} SC + 50 mg L^{-1} MCP at different buffer according to the method mentioned in experimental work is depicted in Fig.(3). It was shown the absobances increase with increasing pH and reached a maximum at pH 1.8 . thereafter, the absorbance sharply decreased due to completely disociation of the complex at higher than pH 1.8. Thus the pH 1.8 was selected as optimum for complete formation of ion-association complex.

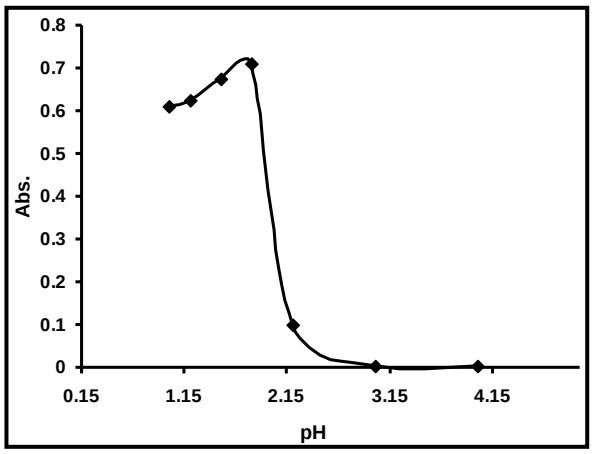


Fig. (3) Effect of pH on the formation of the SC-MCP complex (45 mg L^{-1} SC + 50 mg L^{-1} MCP + 5 ml at different pH) at specified procedure, blank $CHCl_3$.

- **Effect of MCP Concentration**

The effect of MCP concentration was studied by measuring the absorbance of the solutions containing a fixed concentration of SC (45 mg L^{-1}) and different amounts of MCP (5- 60 mg L^{-1}) at optimum pH. It was found that the absorbance of SC-MCP complex increases linearly as the concentration of MCP increases and then slightly decreases after 20 mg L^{-1} (Fig 4). Consequently, the optimum concentration of MCP of 20 mg L^{-1} was selected for complete complex formation.

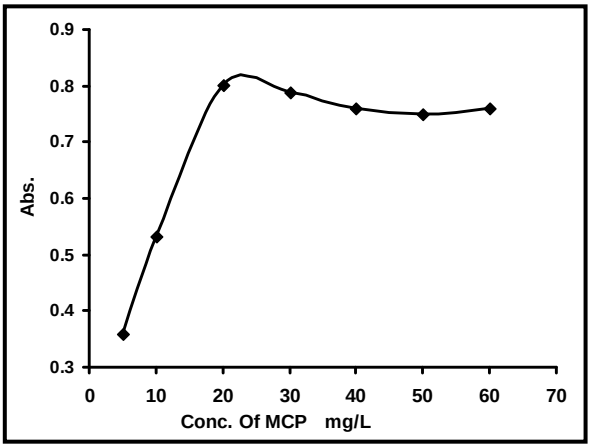
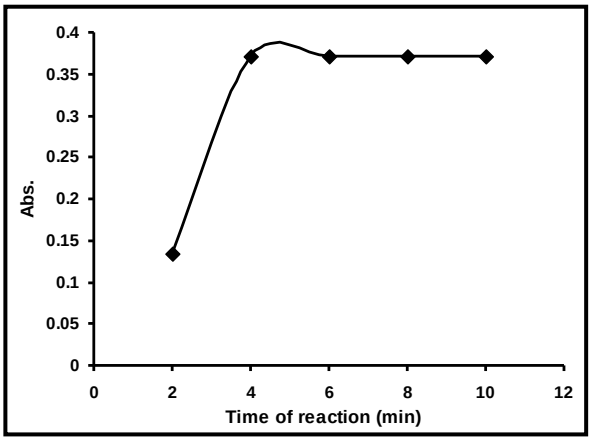


Fig.(4) Effect of concentration of MCP on the determination of SC (45 mg L^{-1} SC + different concentration of MCP) at optimum pH by specified procedure, blank $CHCl_3$.

- **Effect of Reaction Time**

Fig.(5) shows the effect of reaction time on the formation of complex (containing 45 mg L^{-1} SC + 20 mg L^{-1} MCP and other conditions were fixed) before the extraction process at room temperature. It was shown that the absorption signal increases rapidly with the reaction time and approached a plateau at 4 min. Therefore, a reaction time at 4 min was selected.



Fig(5) Effect of the reaction time on the formation of the SC-MCP complex (45 mg L^{-1} SC + 20 mg L^{-1} MCP) at optimum pH, blank $CHCl_3$.

- **Effect of Extraction Time**

The time of shaking for complete extraction of ion-association complex (containing 45 mg L^{-1} SC + 20 mg L^{-1} MCP and other conditions were fixed) was studied and found that the absorbance of the extract remains constant between 1.5-4 min (Fig.6). Thus 1.5 min was chosen as an optimum value throughout the experiments.

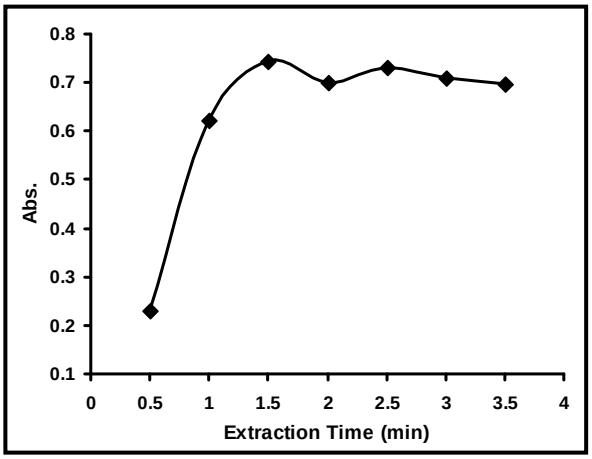


Fig.(6) Effect of extraction time for the SC-MCP complex (45 mg L^{-1} SC + 20 mg L^{-1} MCP) at optimum pH, blank $CHCl_3$.

Extraction efficiency

Table (1) shows molecular absorbance values for the extracted complex of SC with MCP after the first and second extraction of the aqueous phase. The extraction efficiency (E%) was found to be 97.29 and the distribution coefficient (D=59.83) was achieved.

Table (1)
Absorbencies of complex after the first and second extraction.

SC ($mg.L^{-1}$)	*MCP ($mg.L^{-1}$)*	*pH*	*A_1 (Ex. No.1)*	*A_2 (Ex. No.2)*	*A_o Blank*
45	20	1.8	0.902	0.047	0.006

Structure of the complex

Molar ratio method was employed to elucidate the composition of SC-MCP complex formed at optimal conditions. Fig.(7) revealed that a 1:1 (drug-MCP) are formed at λ_{max} 410 nm.

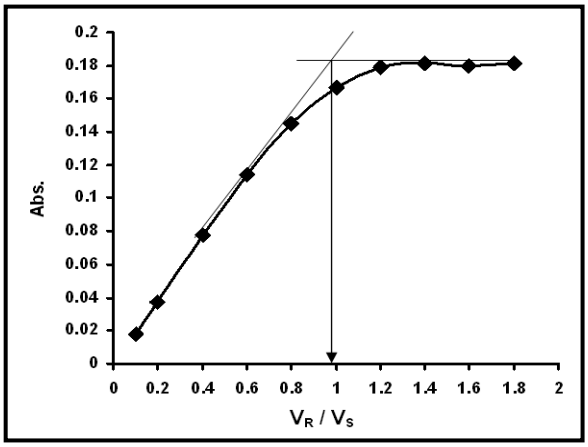
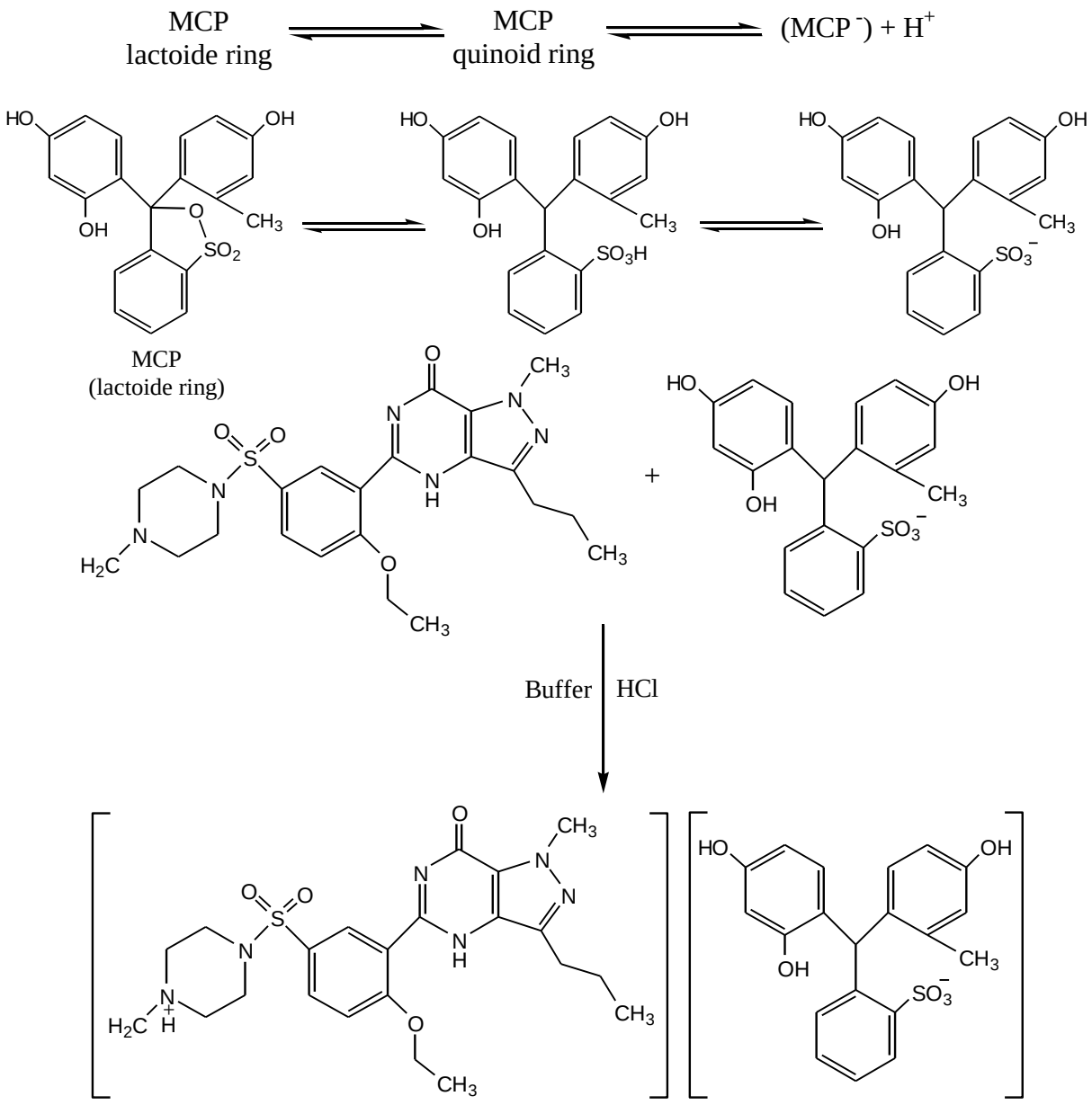


Fig. (7) Mole ratio method for the compositions of SC-MCP complex (V_R = volume of MCP, V_S = volume of SC).

Since the mole ratio is 1:1, it could be expected that the SC-MCP complex with two oppositely charged ions, behaves as a single unit held together by an electrostatic force of attraction [5]. Thus the probable mechanism of formation of the ion-pair complex is shown in the following.



Ion-association complex (Orange)

Calibration Graph

Under the optimum experimental conditions described previously, linearity, detection limit, molar absorptivity, and Sandell's sensitivity were determined. Beer's law was obeyed in the concentration range 3-70 mg L^{-1} of SC at wave length 410 nm. The results are summarized in Table (2).

Table (2)

Analytical characteristics of result.

λ_{max}(nm)	Linearity ($mg.L^{-1}$) SC	D.L.* ($mg.L^{-1}$) (n=11)	D.L.T** ($mg.L^{-1}$)	Sandell sensitivity ($\mu g.cm^{-2}$)	ε ($L.mol^{-1} cm^{-1}$)
410	**3-70**	**0.13**	**0.218**	**0.0452**	**7.86×10^3**

* *Experimental detection limit*

** *Theoretical detection limit*

The direct calibration graph was statistically evaluated which gave the coefficient of determination (R^2) of 99.86%, suggests statistically valid. This fitted linear calibration model was used to estimate the SC concentration in the drug samples which appears justified, on statistical basis. The statistical data are given in Table (3).

Table (3)
Statistical evaluation for the direct calibration graph.

Regression Equation y=bx+a	*Corr. Coef. (r)*	*t- test statistic*	*Tabulated t- test two tailed (n-2) 95% C.I*	*Conf. Limit. for the slope b* $b \pm t_{sb}$	*Conf. Limit for the intercept a* $\pm t_{sa}$
y= 0.019x + 0.017	0.9993	96.33	2.160	0.01906 ± 0.0159	0.01722 ± 0.0065

The accuracy in term of recovery percent and precision were achieved by spiking of 5, 30 and 60 mg L^{-1} using the recommended procedure previously mentioned under standard additions. The results were shown in Table (4). These data indicate that the visible spectrometric determination of SC is not highly effected by the presence of other constituents in the drug sample.

Table (4)
Shows the Recovery%, relative standard deviation (RSD %) and relative error ($E_{rel\%}$).

Amount of SC taken (mg L^{-1})	*Amount of SC found (mg L^{-1})*	*%Rec.*	*%Erel.*	*%RSD (n = 5)*	*Mean %Rec. ±S.D*	*Mean %Erel.*
5	4.9	98	-2	5.27	100.2 ±0.42	0.18
30	30.6	102	2	3.60	---	---
60	60.33	100.55	0.55	1.55	---	---

Determination of SC in Pharmaceuticals

The proposed method was applied for the assay of SC in tablets by using direct calibration and standard additions procedures (Fig.8) under optimum conditions. The SC was determined by measuring the absorbance the complex extracted as a result of the reaction of SC present in the pharmaceutical preparation with MCP.

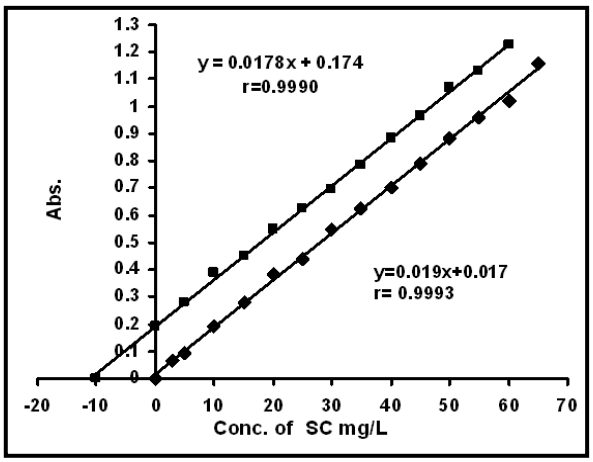


Fig. (8) Determination of SC in pharmaceuticals by using direct and standard additions procedures.

The results for the determination of SC are summarized in Table (5).

Table (6)
Determination SC in sample of pharmaceutical preparations by the proposed method.

Tablet	*Manufac-turer*	*Labeled quantity (mg/unit)*	*Amount Found (mg)*	*Rec. %*	*RSD% (n = 5)*	*E_{rel}% (n = 5)*
VeGon	Atlantis life sciences India	100	92.34	92.34	2.80	-7.66
Excegra	Excel life science Londn U.K	100	99.88	99.88	2.78	- 0.12
KAM-GRA	Ajanta pharma limited India	50	49.55	99.1	3.15	-0.9

It can also be noted from (Fig.8), that the ratio of the slopes of the direct calibration and standard additions is found to be one, which indicates that the interferences resulting from drug constituents are insignificant using the proposed procedure. Thus, it is possible to use direct calibration procedure for the determination of SC in drugs without need the standard additions method which requires more effort, more amount of sample and time-consuming. This is also support the specificity of the proposed method, indicating that the excipients did not interfere with the analysis of SC.

Conclusions

The proposed method is simple, accurate, precise, sensitive and specific and can be used for routine quality control in both pure and

pharmaceuticals without fear of interference. The results show that the quantity of SC in tablets are in a good agreement with given labeled quantity. Furthermore, due to its low detection limit the proposed method could be applied for the assay of SC in biological samples too.

Acknowledgement

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الخلاصة

جرى استحداث طريقة سريعة وبسيطة لتقدير دواء السيلدينافيل في المادة النقية والمستحضرات الصيدلانية. تستند هذه الطريقة على تكوين معقد التجمع الايوني بين الدواء والميتاكريسول الارجواني في محلول منظم حامضي الذي جرى استخلاصه في الكلوروفورم وقياسه كميا عند الطول الموجي الاعظم 410 نانوميتر. دلت النتائج من خلال تحليل الارتداد لمنحني المعايرة بان المدى الخطي الذي يطيع قانون بير كان 3–70 ملغم/ لتر مع حد كشف بمقدار 0.13 ملغم/لتر وحساسية ساندل مقدارها 0.0452 مكغم/ سم$^{-2}$. كما دلت النتائج بان ضبط الطريقة محسوبا على اساس الاسترداد بالمئة كانت بمدى 100.2± 0.42 % في عينات الدواء. وبالنظر لبساطة وسرعة الطريقة المقترحة نوصى استخدامها لاغراض السيطرة النوعية الدورية في تقدير الدواء بصورته النقية وفي المستحضرات الصيدلانية.