

Original Article

VALIDATED SPECTROPHOTOMETRIC METHOD TO DETERMINE VARDENAFIL AND SILDENAFIL IN PHARMACEUTICAL FORMS USING POTASSIUM IODIDE AND POTASSIUM IODATE

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ABSTRACT

Objective: To develop and validate simple, sensitive, precise and free of organic solvents method for the determination of sildenafil (SIL) and vardenafil (VAR) in bulk and pharmaceutical formulation.

Methods: The method is based on the reaction of studied drugs with a mixture of potassium iodide and potassium iodate in an aqueous medium at (25±2 °C) to form yellow coloured triiodide ions (I₃⁻) within 45 min. The reaction is followed spectrophotometrically by measuring the absorbance at 288, 351 nm and 285, 351 nm for sildenafil and vardenafil respectively.

Results: The effects of analytical parameters on the reported systems were investigated. Beer's law of SIL was obeyed in the range of (0.4-12) µg ml⁻¹ and (0.6-16) µg ml⁻¹. Molar absorptivity was found to be (67.659 ×10³) l mol⁻¹/cm and (37.955×10³) l mol⁻¹/cm at 288 nm, 351 nm respectively. Beer's law of VAR was obeyed in the range of (0.2-13) µg/ml and (0.5-40) µg/ml. Moreover, molar absorptivity's were found to be (68.719 ×10³) l mol⁻¹ cm⁻¹ and (26.691×10³) l mol⁻¹ cm⁻¹ at 285 nm, 351 nm respectively.

Conclusion: The proposed method has been applied to determine the components in dosage forms with an average recovery of 98.15% to 103.45% and the results have been found in good agreement with those results obtained by the reference methods.

Keywords: Sildenafil citrate, Vardenafil hydrochloride, Spectrophotometry, Potassium iodide, Potassium Iodate, Triiodide ion

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INTRODUCTION

Erectile dysfunction (ED) is one of the most common chronic diseases affecting men and its prevalence increases with aging. It is also the most frequently diagnosed sexual dysfunction in the older male population. Treatment of erectile dysfunction is based on phosphodiesterase type 5 (PDE-5) inhibitors including sildenafil (SIL), and vardenafil (VAR). PDE-5 inhibitors have high efficacy and safety rates, even in difficult-to-treat populations such as patients with diabetes mellitus [1]. Sildenafil citrate was the first drug approved for the treatment of ED in 1998. The United States food and drug administration (FDA) approved tadalafil and vardenafil hydrochloride in 2003 [2].

There are several studies in medical literature reporting the determination of sildenafil citrate in pharmaceuticals, plasma samples, herbal drugs or dietary supplements using liquid chromatography method [1-7], electroanalytical methods [8, 9] gas chromatography [10], Thin layer chromatography [11], capillary electrophoresis [12] and capillary chromatography [13-15]. Many spectrophotometric Methods have also been reported [16-22].

As same as, determination of VAR in bulk, tablet dosage forms, and biological fluids were analyzed using different analytical systems, including HPLC [3, 23-25], capillary electrophoresis [26] capillary chromatography [27] LC-MS [28], and spectrophotometric methods [29, 30]. In the previous studies, the spectrophotometric determination of SIL and VAR depend on UV spectrophotometric method, oxidation of the drug and complex formation. Most of these methods demand organic solvents or organic reagents, unlike the proposed method.

The aim of the present study was to report a new, simple, and environment-friendly method to determination SIL and VAR as raw materials and in pharmaceutical preparations.

MATERIALS AND METHODS

Apparatus

Uv-visible spectrophotometer (JASCO, model V650, Japan) with 1.00 cm quartz cells. Ultrasonic processor (Powersonic, model 405, Korea) was used to sonicate the sample solutions. Adjustable micro-pipettes covering a volume range from 2 to 5000 µl (ISO-LAB, Germany), used for the preparation of the experimental solutions. Analytical balance (Sartorius, model 2474, Germany). pH meter (CRISON, GLp21/Eu, Spain).

Chemicals and reagents

Pharmaceutical grade sildenafil (99%) and vardenafil (99.5%) were received from XUHANG, CHINA. Potassium iodate and potassium iodide (Panreac, Germany). All reagents and solvents were of analytical grade. Stock standard solution (1 mg ml⁻¹) of SIL and VAR was prepared by dissolving 25 mg from each of SIL and VAR in double distilled water and diluting to 25 ml with double distilled water. 0.15 M of potassium iodate and 0.2 M of potassium iodide solutions were prepared by dissolving the accurately weighed amount of the pure solid in double distilled water. All other chemicals and reagents were of analytical grade and all solutions were prepared with double distilled water. All solutions are stable for a period of 2 d when stored at (4 °C).

General procedure

Increasing volumes of SIL or VAR working standard solution were transferred into series of 10 ml volumetric flasks that contain 2.5 ml of KI (0.2 M) and 1 ml of KIO₃ (0.15 M) for SIL and 3 ml of KI (0.2 M) and 1.0 ml of KIO₃ (0.15M) for VAR. The volume was made up to the mark with distilled water and the absorbance was measured after 45 min at 288, 351 nm and 285, 351 nm against a similar reagent blank for SIL and VAR respectively. The standard calibration plot was prepared to calculate the amount of the analyzed drug in bulk samples. All measurements were carried out at room temperature (25±2 °C).

Procedure for pharmaceutical formulations

Twenty individual tablets were weighed and pulverized carefully. An accurately weighed amount of the powder equivalent to 50 mg of SIL or VAR was transferred into 50 ml volumetric flask and dissolve in 50 ml of water. The content of the flask was sonicated for 30 min then diluted to the volume with water. A portion of this solution was centrifuged for 15 min at 5000 rpm then a suitable volume of the supernatant was transferred into 10 ml volumetric flask, and the procedure was continued to use for the analysis of SIL/VAR by the spectrophotometric method after 45 min.

RESULTS AND DISCUSSION

Absorption spectra

Iodide ions convert to free iodine in an acidic medium of VAR or SIL solution, the acidity comes from HCl or H₃Cit, then free iodine reacts with a surplus of iodide ions to form yellow complex from triiodide (I₃⁻) (fig. 2, 3) [31].

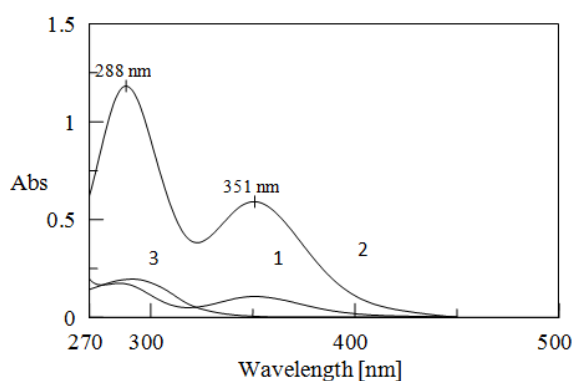
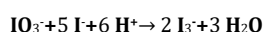


Fig. 1: Absorption spectra of (1) reagent blank against distilled water, (2) SIL (12.0 µg ml⁻¹)+2.5 ml of 0.20 M KI+1.0 ml of 0.15M KIO₃ against reagent, Blank, (3) SIL against distilled water

Optimization of reaction conditions

The optimum conditions for the development of method were established by varying one parameter at a time and keeping the others fixed and observing the effect produced on the absorbance of the coloured products.

A volume of 2.5 ml of 0.2 M KI, 1.0 ml of 0.15 M KIO₃ and 3.0 ml of 0.20 M KI, 1.0 ml of 0.15 M KIO₃ were found to be optimum for

maximum colour development for determination of SIL and VAR respectively. Since the absorbances were found to be maximal at the mentioned volumes. The laboratory temperature (25±2 °C), and the time of reaction was 45 min to obtain the optimum absorbance for all the experiments.

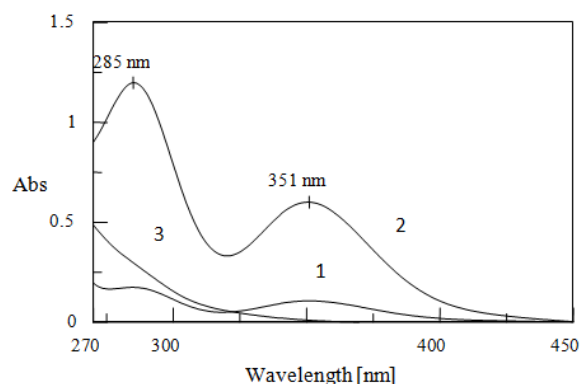


Fig. 2: Absorption spectra of (1) reagent blank against distilled water, (2) VAR (13.0 µg ml⁻¹)+3 ml of 0.20 M KI+1.0 ml of 0.15 M KIO₃ against reagent, Blank, (3) VAR against distilled water

Analytical method validation

Linearity

At described experimental conditions for determination of SIL and VAR, standard calibration curves with good linearity were obtained. The molar absorptivities, detection limits, and limits of quantification were calculated. The high molar absorptivities of the resulting coloured product indicate the high sensitivity of the method. Values of some analytical characteristics for proposed procedures were shown in table 1.

Accuracy and precision

The accuracy and precision of the proposed procedures were carried out by five determinations at several different concentrations for SIL and VAR. Percentage relative standard deviation (RSD %) as precision and percentage recovery as an accuracy of the suggested procedures were calculated and showed in table 2. The values of relative standard deviations for different concentrations of drugs determined from the calibration curves. These results of accuracy and precision show that the proposed procedures have good repeatability and reproducibility. The proposed method is selective to the assay of SIL and VAR in the presence of various excipients.

Table 1: Analytical parameters of spectrophotometric methods

parameters	SIL		VAR	
	288 nm	351 nm	285 nm	351 nm
Linear rang µg. ml ⁻¹	0.4-12	0.6-16	0.2-13	0.5-40
ε L mol ⁻¹ cm ⁻¹	67.659×10 ³	37.955×10 ³	68.719×10 ³	26.691×10 ³
Detection limit µg. ml ⁻¹	0.07	0.09	0.04	0.08
Limit of quantification µg. ml ⁻¹	0.25	0.3	0.13	0.25
Regression equation	*(A = m C+b)			
	m=0.099	m=0.052	m= 0.109	m= 0.044
	b= 0.002	b= 0.011	b= 0.012	b= 0.005
Correlation coefficient	0.9996	0.9988	0.9985	0.9994

*With respect to A=mC+b, where C is the concentration (µg. ml⁻¹) and A is the absorbance.

Application to the pharmaceutical dosage forms

The proposed procedures were applied to determine the studied substances in their pharmaceutical formulations. The results in table 3 indicate the high accuracy and precision. As can be seen from table 3,

the proposed method has the advantages of being virtually free from interferences by excipients and common degradation products. The results obtained were compared statistically by the student's t-test (for accuracy) and the variance ratio F-test (for precision) with those obtained by the reference methods [3, 23] on samples of the

same batch (table 3). The values of t-and F-tests obtained at 95% confidence level and four degrees of freedom did not exceed the

theoretical tabulated value indicating that no significant difference between the proposed method and references method.

Table 2: Accuracy and precision of the determination of SIL and VAR in bulk powder by the proposed method

Drug	λ_{max} , nm	Mg/ml			RSD***%	Recovery%
		Taken	Found	Found \pm S. D**		
SIL	288	0.4	0.403	0.403 \pm 0.015	3.69	100.80
		2	2.014	2.014 \pm 0.039	1.94	100.70
		8	8.056	8.056 \pm 0.113	1.40	100.70
	351	12	12.130	12.130 \pm 0.140	1.15	101.08
		0.6	0.595	0.595 \pm 0.025	4.21	99.16
		4	4.067	4.067 \pm 0.112	2.75	101.67
		10	10.044	10.044 \pm 0.192	1.91	100.44
		16	16.050	16.050 \pm 0.250	1.56	100.31
		285	0.2	0.207	0.207 \pm 0.207	4.40
VAR	285	1	1.026	1.026 \pm 0.047	4.58	102.60
		8	8.132	8.132 \pm 0.130	1.60	101.65
		13	12.950	12.950 \pm 0.209	1.61	99.615
	351	0.5	0.501	0.501 \pm 0.021	4.26	100.29
		4	4.092	4.092 \pm 0.125	3.05	102.30
		20	19.631	19.631 \pm 0.228	1.16	98.15
		40	40.307	40.307 \pm 0.363	0.90	100.76

*Average of five determinations, **Standard deviation, ***Relative standard deviation.

Table 3: Determination of SIL and VAR in their pharmaceutical preparations using the proposed and reference methods

Formula	Drug	Claim (mg/tab)	Found*% \pm SD*		Reference method [3]
			Proposed method λ_{max} , nm	Reference method [3]	
VIGRAVID	SIL	25	288	25.14 \pm 1.17	25.19 \pm 0.52
			351	25.06 \pm 1.92	25.19 \pm 0.52
		50	50.14 \pm 0.90	50.75 \pm 1.45	50.33 \pm 0.92
	351	100	101.98 \pm 0.49	101.15 \pm 1.92	101.35 \pm 0.89
			285	9.93 \pm 0.67	9.98 \pm 1.97
		20	19.86 \pm 1.17	20.17 \pm 1.03	20.01 \pm 0.40
FASTFIX	VAR	10	285	9.93 \pm 0.67	9.963 \pm 0.72
			351	9.98 \pm 1.97	9.963 \pm 0.72
		20	19.86 \pm 1.17	20.17 \pm 1.03	20.01 \pm 0.40
	351	10	19.86 \pm 1.17	20.17 \pm 1.03	20.01 \pm 0.40
			285	9.93 \pm 0.67	9.98 \pm 1.97
		20	19.86 \pm 1.17	20.17 \pm 1.03	

* Average of five determinations, (four degrees of freedom), At 95% confidence level-value is 2.776 and F-value is 6.26, **Supplied by NCPI products, Syria, ***Supplied by UNIPHARMA products, Syria.

DISCUSSION

In this study, the developed spectrophotometric method is free of organic solvents [16-20, 22, 29], rapid, and has more sensitivity than previous methods [16-20, 22, 29, 30]. The proposed method can be carried out without any organic solvents or reagents in contrast with many previous spectrophotometric methods.

All previous reasons make this method easier and cheaper from other spectrophotometric methods.

A comparative summarized study between the proposed methods and previous spectrophotometric methods for determination of SIL and VAR has been provided in table 4 and table 5 respectively.

Table 4: Comparison of the proposed methods with the existing spectrophotometric methods for the determination of Sildenafil citrate

Reagent	Spectrophotometric Method	Solvent	Temp. °C	Concentration rang. μ g/ml	ϵ l/mol/cm	Reference
iodine	direct	dichloroethane	25 \pm 1	15.0-160	3.75 \times 10 ³	[16]
TCNQ	direct	acetonitrile	50 \pm 2	15.0-220	2.58 \times 10 ³	[16]
DDQ	direct	methanol	50 \pm 2	20.0-260	2.41 \times 10 ³	[16]
TCNE	direct	acetonitrile	50 \pm 2	10.0-210	3.05 \times 10 ³	[16]
TNF	direct	dichloroethane	60 \pm 2	15.0-240	2.25 \times 10 ³	[16]
chloranilic acid	direct	acetonitrile	60 \pm 2	20.0-180	3.26 \times 10 ³	[16]
chloranil	direct	acetonitrile	60 \pm 2	28.0-150	3.42 \times 10 ³	[16]
bromanil	direct	methanol	60 \pm 2	15.0-170	2.90 \times 10 ³	[16]

BCG	extractive	chloroform	25±2	1.2-25.0	1.58×10 ⁴	[17]
CCR	extractive	chloroform	25±2	1.5-60.0	9.79×10 ³	[17]
chromotrope 2B	extractive	Methylene chloride	25±2	3.3-87.0	1.02×10 ⁴	[18]
chromotrope 2R	extractive	Methylene chloride	25±2	3.3-96.0	8.30×10 ³	[18]
3-phenylazo-6-o-carboxyphenylazo-chromotropic acid	extractive	Methylene chloride	25±2	5.0-115.0	6.83×10 ³	[18]
bis-3,6-(o-hydroxyphenylazo)-chromotropic acid	extractive	Methylene chloride	25±2	2.5-125.0	5.42×10 ³	[18]
bis-3,6-(p-N,N-dimethylphenylazo)-chromotropic acid	extractive	methylene chloride	25±2	8.3-166.7	3.35×10 ³	[18]
3-phenylazo-6-o-hydroxyphenylazo-chromotropic acid	extractive	Methylene chloride	25±2	0.8-15.0	2.32×10 ⁴	[18]
BTB	extractive	chloroform	40±2	1.0-40.0	20.12×10 ³	[19]
PBP	extractive	chloroform	25±2	1.0-50	44.40×10 ³	[19]
EPPR	extractive	chloroform	25±2	3.0-70.0	24.00×10 ³	[19]
MCP	extractive	chloroform	25±2	3.0-70.0	7.86×10 ³	[20]
BCG	direct	dichloromethane	25±2	1.3-50.0	2.28×10 ⁴	[22]
BTB	direct	dichloromethane	25±2	0.8-27.0	3.05×10 ⁴	[22]
Proposed method						
KI+KIO ₃ (288 nm)	direct	water	25±2	0.4-12	6.7659×10 ⁴	
KI+KIO ₃ (351 nm)	direct	water	25±2	0.6-16	3.7955×10 ⁴	

Table 5: Comparison of the proposed methods with the existing spectrophotometric methods for the determination of vardenafil hydrochloride

Reagent	Spectrophotometric method	Solvent	Temp. °C	Concentration rang. µg/ml	ε l mol/cm	Reference
BCG	extractive	chloroform	25±2	5-60	3.755×10 ⁴	[29]
acetaldehyde+ sodium nitroprusside	direct	alkaline medium	25±2	2-20	4.079×10 ⁴	[29]
KMnO ₄	oxidation	alkaline medium	25±2	10-100	4.299×10 ³	[29]
methyl benzothiazolinone hydrazoneHCL	oxidation	acidic medium	25±2	4-40	3.774×10 ⁴	[30]
4-aminoantipyrine+potassium periodate	indirect-oxidation	alkaline medium	25±2	4-60	4.605×10 ⁴	[30]
Proposed method						
KI+KIO ₃ (285 nm)	direct	water	25±2	0.2-13	6.8719×10 ⁴	
KI+KIO ₃ (351 nm)	direct	water	25±2	0.4-40	2.6691×10 ⁴	

We can see this is the unique colorimetric method does not need any organic compound to determine SIL and VAR.

CONCLUSION

The proposed analytical procedures were simple, rapid, accurate and precise, so it can be used for the routine analysis of SIL and VAR in bulk and pharmaceutical formulations. The sample recoveries from all formulations have good agreement with their respective label claims, which suggested non-interference with formulations excipients in the assay. Moreover, the present method is environment-friendly because it does not need any organic reagents or solvents; it is free extractive and very sensitive comparing with other spectrophotometric methods.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

REFERENCES

- Gareri P, Castagna A, Francomano D, Cerminara G, De Fazio P. Erectile dysfunction in the elderly: an old widespread issue with novel treatment perspectives. *Int J Endocrinol* 2014. <http://dx.doi.org/10.1155/2014/878670>
- Codevilla CF, Castilhos TD, Bergold AM. A review of analytical methods for the determination of four new phosphodiesterase type 5 inhibitors in biological samples and pharmaceutical preparations. *Braz J Pharm Sci* 2013;49:1-1.
- Reddy B, Reddy YR. Validation and stability indicating RP-HPLC method for the determination of sildenafil citrate in pharmaceutical formulations and human plasma. *J Chem* 2008;5 Suppl 2:1117-22.
- Tripathi AS, Sheikh I, Dewani AP, Shelke PG, Bakal RL, Chandewar AV, *et al.* Development and validation of RP-HPLC method for sildenafil citrate in rat plasma-application to pharmacokinetic studies. *Saudi Pharm J* 2013;21:317-21.
- Kim J, Ji H, Kim SJ, Lee HW, Lee SS, Kim DS, *et al.* Simultaneous determination of sildenafil and its active metabolite UK-103,320 in human plasma using liquid chromatography-tandem mass spectrometry. *J Pharm Biomed Anal* 2003;32:317-22.
- Tracqui A, Ludes B. HPLC-MS for the determination of sildenafil citrate (Viagra®) in biological fluids. Application to the salivary excretion of sildenafil after oral intake. *J Anal Toxicol* 2003;27:88-94.
- Aboul-Enein HY, HEFNAWY MM. Rapid determination of sildenafil citrate in pharmaceutical preparations using monolithic silica HPLC column. *J Liq Chromatogr Relat Technol* 2003;26:2897-908.
- Berzas JJ, Rodriguez J, Castaneda G, Villaseñor MJ. Voltammetric behavior of sildenafil citrate (Viagra) using square wave and adsorptive stripping square wave techniques: determination in pharmaceutical products. *Anal Chim Acta* 2000;417:143-8.
- Özkan SA, Uslu B, Zuman P. Electrochemical oxidation of sildenafil citrate (Viagra) on carbon electrodes. *Anal Chim Acta* 2004;501:227-33.
- Berzas Nevado JJ, Villaseñor Llerena MJ, Contenido Salcedo AM, Rodríguez Flores J. Development of a capillary gas chromatographic method with flame ionisation detection for the

- simultaneous determination of sildenafil and its N-demethylated metabolite in biological fluids. *J Sep Sci* 2002;25:767-72.
11. Pelander A, Ojanperä I, Sistonen J, Rasanen I, Vuori E. Screening for basic drugs in 2-ml urine samples by dual-plate over pressured layer chromatography and comparison with gas chromatography-mass spectrometry. *J Anal Toxicol* 2003;27:226-32.
 12. Qin W, Li SF. An ionic liquid coating for determination of sildenafil and UK-103,320 in human serum by capillary zone electrophoresis-ion trap mass spectrometry. *Electrophoresis* 2002;23:4110-6.
 13. Flores JR, Nevado JB, Penalvo GC, Diez NM. Development of a micellar electrokinetic capillary chromatography method for the determination of three drugs employed in the erectile dysfunction therapy. *J Chromatogr B* 2004;811:231-6.
 14. Nevado JB, Flores JR, Penalvo GC, Farinas NR. Determination of sildenafil citrate and its main metabolite by sample stacking with polarity switching using micellar electrokinetic chromatography. *J Chromatogr A* 2002;953:279-86.
 15. Nevado JJ, Flores JR, Penalvo GC, Fariñas NR. Micellar electrokinetic capillary chromatography for the determination of Viagra and its metabolite (UK-103,320) in human serum. *Electrophoresis* 2001;22:2004-9.
 16. Amin AS, El-Beshbeshy AM. Utility of certain σ and π -acceptors for the spectrophotometric determination of sildenafil citrate (Viagra). *Mikrochim Acta* 2001;137:63-9.
 17. Dinesh ND, Nagaraja P, Gowda NM, Rangappa KS. Extractive spectrophotometric methods for the assay of sildenafil citrate (Viagra) in pure form and in pharmaceutical formulations. *Talanta* 2002;57:757-64.
 18. Issa YM, El-Hawary WF, Youssef AF, Senosy AR. Spectrophotometric determination of sildenafil citrate in pure form and in pharmaceutical formulation using some chromotropic acid azo dyes. *Spectrochim Acta Mol Biomol Spectrosc* 2010;75:1297-303.
 19. Frag EY, Mohamed GG, Alelaiwi HM. Utility of ion-associate formation reactions for the spectrophotometric determination of sildenafil citrate in pure form and in virecta tablets. *Pharm Anal Acta* 2011;2:2.
 20. Muhamad YH. A simple spectrophotometric assay of sildenafil in pure and pharmaceutical preparations. *J Al-Nahrain University* 2012;15:18-24.
 21. El-Gindy AE, Shokry E, Farouk M, EL-AZIZ L. Validated methods for determination of sildenafil citrate in the presence of its potential impurities. *Int J Biomed Res* 2010;2:262-78.
 22. Sakur AA, Affas S. Direct spectrophotometric determination of sildenafil citrate in pharmaceutical preparations via complex formation with two sulphonphthalein acid dyes. *Res J Pharm Tech* 2017;10:1191-6.
 23. Manisha G, Usha P, Vandana P. Development and validation of RP-HPLC method for estimation of vardenafil in bulk and pharmaceutical formulation. *Am J PharmTech Res* 2013; 3:928-38.
 24. Rao DS, Surendranath KV, Radhakrishnanand P, Suryanarayana MV, Raghuram P. A stability indicating LC method for vardenafil HCl. *Chromatographia* 2008;68:829-35.
 25. Di Y, Zhao M, Nie Y, Wang F, Lv J. A high-performance liquid chromatography: chemiluminescence method for potential determination of vardenafil in dietary supplement. *J Anal Methods Chem* 2011;20:2011.
 26. Idris AM, Alnajjar AO. Multi-response optimization of a capillary electrophoretic method for determination of vardenafil in the bulk drug and in a tablet formulation. *Acta Chromatogr* 2007;1:19-97.
 27. Flores JR, Nevado JB, Penalvo GC, Diez NM. Development of a micellar electrokinetic capillary chromatography method for the determination of three drugs employed in the erectile dysfunction therapy. *J Chromatogr B* 2004;811:231-6.
 28. Gratz SR, Flurer CL, Wolnik KA. Analysis of undeclared synthetic phosphodiesterase-5 inhibitors in dietary supplements and herbal matrices by LC-ESI-MS and LC-UV. *J Pharm Biomed Anal* 2004;36:525-33.
 29. Reddy TV. Spectrophotometric quantification of vardenafil in bulk and tablet. *Int J Chem Biol Sci* 2015;3:185-92.
 30. Kumar AV, Reddy TV, Sekharan C. Spectrophotometric analysis of vardenafil in tablet dosage forms by using electrophilic coupling reagents. *Anal Bioanal Chem* 2016;3:29-39.
 31. Khateeb M, Elias B, Al Rahal F. Validated spectrophotometric method to assay of B6 and B3 vitamins in pharmaceutical forms using potassium iodide and potassium iodate. *Int Lett Chem Physics Astronomy* 2015;60:113-9.