DETERMINATION OF VARDENAFIL IN PURE AND DOSAGE FORMS BY SPECTROPHOTOMETRY**

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Three simple, sensitive, precise, and rapid spectrophotometric methods are developed and optimized for the assay of vardenafil in pharmaceutical formulations. The procedures are ascertained on the oxidation of vardenafil with a slight excess of N-bromosuccinimide (NBS), and the unreacted oxidant is estimated by evaluating the amount of the unconsumed NBS by reacting with a fixed amount of safranin (method A), crystal violet (method B), and aniline blue (method C) in an acidic medium by measuring the absorbance at 530, 600, and 610 nm, respectively. In all the methods, the amount of NBS consumed corresponds to the amount of the drug. Beer's law range, Sandell's sensitivity, and the corresponding molar absorptivity values of 1.1104×10^4, 1.0305×10^4, and 6.217×10^3 L/mol · cm for methods A, B, and C are calculated. The limits of detection and quantification are evaluated. The methods are also evaluated statistically by means of Student's t-test and F-test. The results show excellent agreement and insignificant difference between the proposed methods and the reference method. The developed methods are successfully applied to the assay of Vardenafil in pure and dosage forms, and no interference is observed from common excipients present in pharmaceutical formulations.

Keywords: *drug research, N-bromosuccinimide, redox reaction, vardenafil, UV-Vis spectroscopy.*

СПЕКТРОФОТОМЕТРИЧЕСКИЙ МЕТОД ОПРЕДЕЛЕНИЯ ВАРДЕНАФИЛА В ЧИСТОМ ВИДЕ И ЛЕКАРСТВЕННЫХ ФОРМАХ

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Для анализа варденафила в фармацевтических препаратах разработаны и оптимизированы три спектрофотометрических метода. Варденафил окисляется небольшим избытком N-бромсукцинимида (NBS), и непрореагировавший оксидант оценивается путем определения количества неизрасходованного NBS в реакциях с фиксированным количеством сафранина (метод А), кристалличе-

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ского фиолетового (метод B) и анилинового синего (метод C) в кислой среде по измерениям оптической плотности при 530, 600 и 610 нм соответственно. Для всех методов количество потребляемого NBS соответствует количеству лекарственного препарата. Рассчитаны диапазон выполнимости закона Бера, чувствительность Сэнделла и коэффициенты молярной экстинкции 1.1104×10^4, 1.0305×10^4 и 6.217×10^3 л/моль · см для методов A, B и C соответственно. Оценены пределы обнаружения и количественного определения. Методы также оценены статистически с помощью t-критерия Стьюдента и F-критерия. Результаты показывают отличное совпадение и незначительное различие между эталонным и предложенными методами. Разработанные методы успешно применены для анализа варденафила в чистом виде и лекарственных формах.

Ключевые слова: *лекарственное средство, N-бромсукцинимид, окислительно-восстановительная реакция, варденафил, УФ-видимая спектроскопия.*

Introduction. Vardenafil (VAR) is a benzene sulfonamide derivative and selective potent inhibitor of specific phosphodiesterase type 5 with vasodilatory activity. It is chemically designated as a sulfa group of 4-ethoxy-3-(5-methyl-7-propylimidazo[5,1-f][1,2,4]triazin-4(1H)-one-2-yl) benzenesulfonic acid and the secondary amino group of 4-ethylpiperazine. It is used in the treatment of male erectile dysfunction (impotence) and pulmonary arterial hypertension (PAH) [1–3]. It is also used as an effective therapy for cardiovascular diseases.

A few methods have been reported for VAR in pharmaceutical dosage forms and in biological fluids, including atomic emission spectrometry [4], electro kinetic capillary chromatography [5], electrochemical method [6], gas chromatography [7, 8], LC-MS [9], ultra-performance liquid chromatography [10], high performance liquid chromatography [11], RP-HPLC [12], UV-chemometric and HPLC-QbD [13], RP-HPLC, and UV-spectroscopy [14], RP-UPLC [15], pharmacokinetic study [16], extractive spectrophotometry [17], spectrophotometry [18–23], and thin layer chromatography [24].

Most of the aforementioned methods are complex, time-consuming, inaccessible for routine drug monitoring, and require expensive equipment. In contrast, visible spectrophotometry is one of the most convenient analytical techniques due to its simplicity and reasonable sensitivity with significant economic advantages, finding wide application in all quality control laboratories and pharmaceutical industries in the examination of various groups of drugs.

Reviewing the literature, we have noted that few papers have been published on the problem of the determination of VAR by spectrophotometric methods, and of those methods reported they suffer from one or another drawback such as drastic experimental conditions, utility of organic solvents, longer standing time, poor sensitivity, and a narrow linear range. Therefore, the development and validation of new spectrophotometric methods for the determination of VAR that can overcome the drawbacks of the presented methods are essential.

The present work illustrates new, sensible, simple, cost-effective, and selective spectrophotometric methods for the estimation of VAR in pharmaceutical dosage forms, and is a continuation of our research on biologically important drugs in the pharmaceutical area such as Cephalosporins [25], and Dobutamine Hydrochloride [26]. We propose new sensitive and precise spectrophotometric methods for the development and validation of VAR in pharmaceutical dosage forms by the efficacy of NBS and dyes.

N-Bromosuccinimide (NBS) is a well-known oxidizing agent. For the estimation of NBS, we use three dyes – Safranin, Crystal violet, and Aniline blue – owing to their high absorptivity. Previously, we have revealed the applicability of NBS as a significant reagent for the assay of drugs–namely, Mirabegron [27], Dexmedetomidine hydrochloride [28], Phenylephrine Hydrochloride, and Pyridoxine Hydrochloride [29]. NBS is also used in the determination of several drugs such as Metaprolol tartrate [30] and Sumatriptan succinate [31]. The present work extends the efficacy of NBS as an oxidimetric reagent for the assay of VAR in pharmaceutical formulations. The present investigation aims to develop sensitive and cost-effective spectrophotometric methods for the estimation of VAR in pure and pharmaceutical dosage forms based on the redox reaction of the drug with an excess of NBS and the determination of the unreacted oxidant by the decrease in the absorbance of the dyes, Safranin, Crystal violet, and Aniline blue. The developed methods are more prone than the reported methods, and they are free from such experimental variables, viz., pH, temperature and heating, or the extraction step. These methods can be successfully applied for the assay of the drug in quality control laboratories and it is official in the United States pharmacopoeia (USP) and European pharmacopoeia (EP). The methods were validated according to the ICH guidelines.

Experimental. All the absorbance spectral measurements were made using the double beam BL 198 Bio spectrophotometer (UV-Vis) with 1-cm matched quartz cells with a wavelength range of 190–1100 nm and a spectral bandwidth of 2 nm.

VAR was purchased from Rainbow Pharma Training Labs, Hyderabad, India, and analytical grade safranin, crystal violet, and aniline blue are from S.D. Fine Chemicals PVT. Ltd., Mumbai, India. Also, NBS (Merck, Germany), H_2SO_4 (Ranbaxy fine chemical, India), were used. All of the chemicals used were of analytical reagent grade, and all solutions were freshly prepared.

A stock standard solution containing 10 mg of VAR was prepared by dissolving an appropriate weight of the pure drug in a little amount of methanol and filled up to the mark with distilled water in a 100-mL volumetric flask. Quantitatively, the standard solution was further diluted according to the linearity range.

A stock solution of 0.01% NBS [1-bromo-2,5-pyrrolidinedione], ($C_4H_4BrNO_2$, M. wt. 177.98 g/mol) was freshly prepared by dissolving 10 mg of NBS in a little warm water and diluting to 100 mL of water and standardized [32]. A stock solution of safranin [3,7-diamino-2,8-dimethyl-5-phenylphenazin-5-ium chloride] ($C_{20}H_{19}ClN_4$, M. wt. 350.85 g/mol) was prepared by dissolving 30 mg of the dye in distilled water and diluting with 100 mL of water. A stock solution of crystal violet [Tris(4-(dimethylamino)phenyl)methylium chloride] ($C_{25}H_{30}ClN_3$, M. wt. 407.979 g/mol) was prepared by dissolving 10 mg of dye in distilled water and diluting to 100 mL of water. A stock solution of aniline blue [2-(ammoniométhyl)-3-({4-[(2-sulfonatophenyl)amino]phényl}{4-[(2-sulfonatophényl)imino]-2,5-cyclohexadien-1-ylidene}methyl)benzenesulfonate disodium] ($C_{32}H_{25}N_3O_9S_3Na_2$, M. wt. 737.72374 g/mol) was prepared by dissolving 20 mg of the dye in distilled water and diluting to 100 mL of water. Lastly, a 0.1 M of H_2SO_4 was prepared by diluting 0.27 mL of concentrated acid to 50 mL with distilled water.

Twenty tablets of VAR (Alembic Pharmaceutical Ltd., India**)** were weighed and ground into a fine powder. An accurately weighed quantity containing 10 mg of drug VAR (Staxyn) was dissolved in a small amount of methanol, shaken well for 20 min, sonicated, and filtered. The filtrate was diluted to 100 mL with distilled water in a 100 mL volumetric flask. The resultant solution was further diluted with a requisite amount of water. The drug content in the diluted solution was determined by the assay procedures.

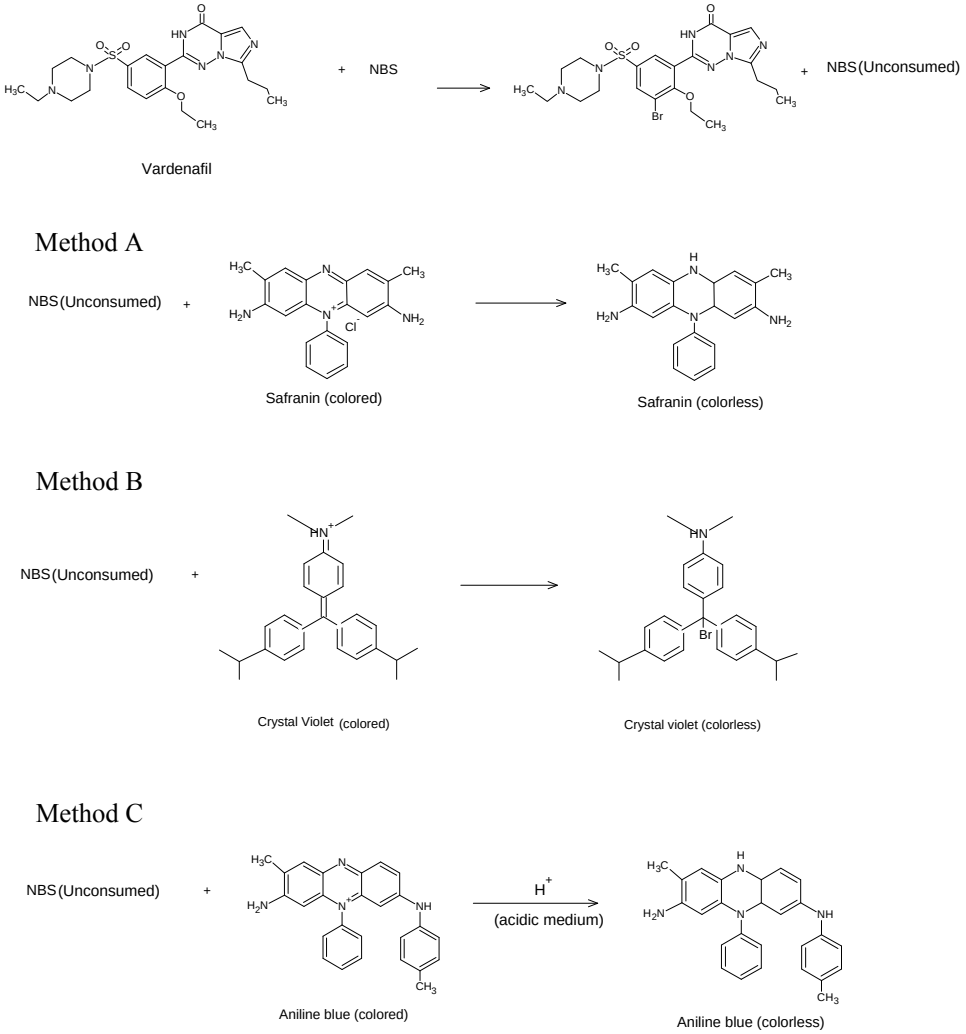
Various aliquots of pure VAR were transferred into a series of 10 mL measuring flasks by means of a micro pipette, which could be diluted quantitatively to obtain 6–36, 6–30, and 12–48 μg/mL for methods A, B, and C, respectively. We added 2.0 mL of 0.01% NBS to each flask, then after 10 min, 0.8 mL of 0.03% safranin dye (method A), 1.2 mL of 0.01% crystal violet (method B), 2.0 mL of 0.1M H_2SO_4, and 0.8 mL of 0.02% aniline blue (method C) were added. The contents were mixed and made up to the volume with deionized water. After that, we measured the absorbance of each solution against the corresponding reagent blank prepared in a similar way in the absence of the drug at λ_{max} = 530, 600, and 610 nm.

Results and discussion. The proposed methods were based on the oxidation of the drug by adding a known excess of the oxidant (NBS) which allowed for the completion of the oxidation process. Then, the oxidation process was confirmed to be complete and the unconsumed NBS was reacted with a fixed quantity of dye – safranin dye (method A), crystal violet (method B), aniline blue (method C) – in an acidic medium with the resulting absorbance measured at 530, 600, and 610 nm, respectively. The decrease in the concentration of NBS upon reacting with a fixed concentration of dyes, which results in an increase in absorbance, is due to the bleaching action of the dyes by NBS, which in turn is related to the drug concentration and caused by the oxidative destruction of the dyes.

The absorption spectra of the reaction product with the drug shows the maximum absorption λ_{max} = 530, 600, 610 nm for methods A, B, and C, respectively. The blank solution showed negligible absorbance at λ_{max} where the drug was tested. The absorption spectra of the reaction product and the corresponding reagent blank for methods A, B, and C are shown in Fig. 1. Beer's law was obeyed in the concentration range 6–36, 6–30, and 12–48 μg/mL for methods A, B, and C, respectively. The curves were found to be linear with good correlation coefficients.

The developed spectrophotometric methods were based on the redox reaction between the drug, dye, and NBS (methods A and B), drug, dye, and NBS in an acidic medium (method C) at room temperature, respectively. NBS acts as a very good oxidizing agent. In all the developed methods, the drug is reacted with a known excess of NBS, and the unreacted oxidant NBS was estimated by reacting with a fixed amount of safranin dye or crystal violet (methods A and B), aniline blue in an acidic medium (method C) followed by the absorption measurement at 530, 600, and 610 nm. The absorbance increased linearly with an increase in the concentration of the drug, when an increasing amount of the drug was added to a fixed amount of 0.01% NBS, and a concomitant decrease in the concentration of NBS occurred. When a fixed amount of the dye

was added to a decreasing concentration of NBS, a concomitant increase in the concentration of the dye was obtained, which in turn is directly proportional to the concentration of the drug. The suggested reaction mechanism is shown in Scheme 1.



Scheme 1. Proposed reaction mechanism of VAR with NBS.

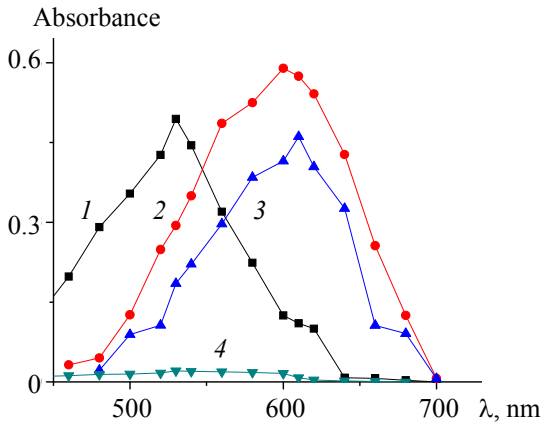


Fig. 1. Absorption spectra of VAR with safranin dye (24 μg/mL) (method A) (1), crystal violet (30 μg/mL) (method B) (2), aniline blue (36 μg/mL) (method C) (3), against reagent blank (4).

The effects of the reagent concentration, time, temperature, stability, the order regarding the addition of reagents with respect to maximum sensitivity, and minimum blank adherence to Beer's law were studied. The optimum conditions were determined by modifying one variable and examining its effect on the absorbance of the dye products.

The effect of the NBS concentration was studied in the range 1.0–4.0 mL. It was found that the maximum absorbance of the coloring products was attained with 2.0 mL of 0.01% NBS. The intensity of color decreases above the upper limit. The order of addition of reagents plays a major role in the formulation of the drug. The maximum color intensity was accomplished by the addition of the drug followed by the dyes.

The thermal effect was examined by heating a blank and a series of sample solutions at different temperatures from 40–80°C in a water bath. It is found that increasing the temperature decreases the color intensity and no reproducible results are obtained. Thus, the maximum color intensity is achieved at room temperature (25±30°C).

The effect of time required for the complete oxidation of the drug was studied at different intervals of time from 5 to 30 min. It was examined that 10 min was required for the oxidation of the drug, after the addition of the dyes; 5–10 min for bleaching. The stability of the color was examined and the resulting drug dye complex was stable for more than 2, 2, and 1 h for methods A, B, and C, respectively.

The reaction was examined by using H_2SO_4, HCl, HNO_3, HCOOH, and CH_3COOH. The optimum results were obtained in a sulphuric acid medium. The variation in the H_2SO_4 concentration pointed out that the invariable absorbance was attained with 0.5–4.0 mL of 0.1 M H_2SO_4 for the drug; thus, the consequent studies were carried out with 2.0 mL of 0.1 M H_2SO_4 for VAR.

The best series of addition was drug-NBS and then the dye for methods A and B and drug-H_2SO_4-NBS-dye for method C under the same experimental conditions. The other series did not give reproducible results.

Method validation. A linear correlation was established between the absorbance at λ_{max} and the concentration of the studied drug. The results were found in the range 6–36, 6–30, and 12–48 μg/mL for methods A, B, and C, respectively. The graphs show negligible intercept and are depicted by the equation $Y = a + bX$, where Y is the absorbance, a is the intercept, b is the slope, and X is the concentration in μg/mL.

Using the method of least squares, the regression analysis of Beer's law was calculated, which revealed an excellent correlation. The optical parameters such as Beer's law limit, molar absorptivity, Sandell's sensitivities, regression equation, and correlation coefficients are as given in Table 1. Beer's law curves of VAR with the dyes are shown in Fig. 2 for methods A, B, and C. The methods were validated according to the current ICH guidelines [33]. Limits of detection (LOD) and quantification (LOQ) were also calculated according to the ICH guidelines using the formulae LOD = 3.3S/b, and LOQ = 10S/b, where S is the standard deviation of blank absorbance values, and b is the slope of the calibration curve. The results are presented in Table 1 and prove the sensitivity of the methods.

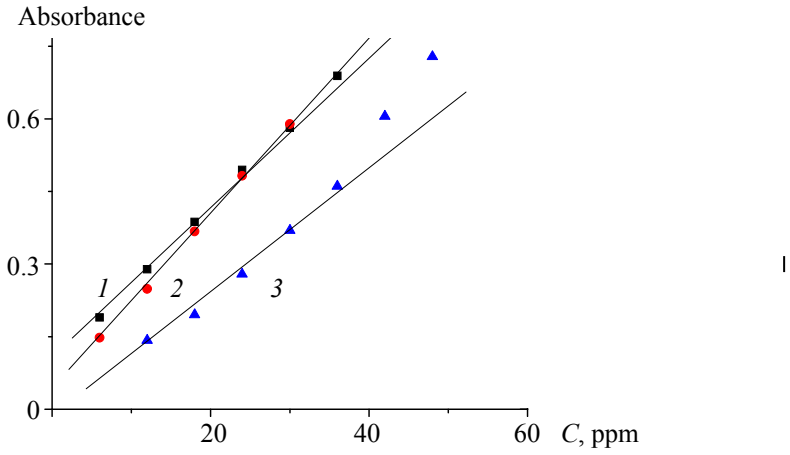


Fig. 2. Beer's law curves of VAR with safranin, crystal violet, and aniline blue method A (1), method B (2), and method C (3).

TABLE 1. Optical Characteristics, Statistical Data and Validation Parameters of the Drug VAR

Optical characteristics	Regression parameters		
	method A	method B	method C
Color	pink	violet	blue
λ_{max}, nm	530	600	610
Beer's law limit (μg/mL)	6–36	6–30	12–48
Molar absorptivity (L/mol · cm)	1.1104×10^4	1.0305×10^4	6.217×10^3
Sandell's sensitivity (μg/cm^2)	0.0439	0.0474	0.07858
Limit of detection [LOD] (μg/mL)	0.2302	0.070	0.0802
Limit of quantification [LOQ] (μg/mL)	0.6977	0.2124	0.2430
Regression equation $Y = BX + A$			
Slope B	0.01657	0.01863	0.0164
Intercept A	0.090420	0.03161	–0.09575
Correlation coefficient r	0.99976	0.99968	0.9900
Relative standard deviation*	0.052	0.0094	0.012

N o t e. X is the concentration of the measured solution in μg/mL and Y is the unit for absorbance.
*Average of five determinations (concentrations of 12, 24, and 36 μg/mL (method A), 12, 18, and 24 μg/mL (method B), and 18, 30, and 42 μg/mL (method C) for VAR, respectively.

Interference studies. To assess the specificity of the methods, the effect of excipients used in the pharmaceutical preparation of the drug was studied by spiking the excipients into the preanalyzed quantity of the drug, as mentioned in Table 2. The tolerance limit was defined as the concentration which gave an error of ±3.0% in the determination of the drug. The comparison of the standard spectra and the spectra from the tablet solution showed that there was no difference in the wavelengths of the maximum absorbance and the maxima/minima. It was concluded that the excipients such as lactose, polyethylene glycol, magnesium stearate, starch, and talc, did not interfere in the analysis.

TABLE 2. Recoveries of the Drug in the Presence of 100 Folds Concentration of Various Additives Used as Excipients in Formulation

Excipients	% Recovery ± %RSD [a]		
	method A [b]	method B [c]	method C [d]
Lactose	98.7±0.4	98.7±0.4	99.6±0.2
Polyethylene glycol	99.8±0.3	99.7±0.2	99.8±0.3
Starch	99.8±0.2	99.8±0.2	99.7±0.3
Talc	99.8±0.2	99.7±0.2	98.9±0.2
Magnesium stearate	99.8±0.3	99.8±0.3	99.7±0.3

[a] Mean ± % R.S.D, $n = 3$. Mean of three determinations.
[b] Concentration of VAR used 18 μg/mL.
[c] Concentration of VAR used 30 μg/mL.
[d] Concentration of VAR used 36 μg/mL.

Accuracy and precision studies. The precision of the proposed methods was calculated in terms of intermediate precision (intra-day and inter-day) at three different concentration levels 12, 24, and 36 μg/mL (method A), 12, 18, and 24 μg/mL (method B), and 18, 30 and 42 μg/mL (method C) in five replicates during the same day and for five consecutive days at the same concentration level. The analytical results obtained from the study are summarized in Table 3. The low values of SD show excellent precision.

Application to formulations. The proposed methods were applied to the assay of VAR in pure and dosage forms. The results achieved by the proposed methods and the reported method [14] for the dosage forms were compared statistically by means of a Student's t-test for accuracy and an F-test for precision at the 95% confidence level. The calculated t- and F-values did not exceed the tabulated values ($t = 2.44$, $F = 5.05$) and indicated an insignificant difference between the proposed methods and the reported method (Table 4).

TABLE 3. Results of Intraday and Inter-day Precision

Formulation	Intra-Day			Inter-Day	
	Amount taken, µg/mL	Amount found, µg/mL	%Recovery±%RSD [a]	Amount found, µg/mL	% Recovery ±%RSD [b]
VAR (A)	12	11.99	99.9±2.2	11.98	99.83±2.0
	24	24.01	100.04±0.56	23.99	99.95±0.48
	36	35.98	99.94±1.24	35.61	98.91±0.92
VAR (B)	12	11.98	99.83±071	12.01	100.0±0.82
	18	18.01	100.05±0.008	17.95	99.72±0.006
	24	23.99	99.95±048	23.91	99.62±0.34
VAR (C)	18	18.01	100±0.86	17.99	99.94±0.79
	30	29.99	99.96±0.70	30.01	100.03±0.71
	42	41.98	99.95±0.24	41.55	98.92±0.19

[a] Mean value of five determinations.
[b] Mean of five determinations performed over a period of five days.

TABLE 4. Results of Analysis (% Recovery ± SD) of Drug VAR (Staxyn)[*] in Pharmaceutical Formulation and Statistical Comparison of the Results with the Reference Method

Method	Proposed method	Reference method [a] (RP-HPLC)
A	99.65±0.99 $t = 0.15$ $F = 2.54$	99.72±0.62
B	99.75±0.51 $t = 0.71$ $F = 1.0$	99.96±0.51
C	99.58±0.79 $t = 0.6$ $F = 1.57$	99.83±0.63

[*] VAR (Staxyn) equivalent to 10 mg/tab (Alembic Pharmaceutical Ltd.) for methods A, B, and C.

[a] Mean of five determinations ± Standard deviation, $n = 5$; the t- and F-values obtained after comparison to the reference method, which have the following theoretical values at the 95% confidence limit $t = 2.44$ and $F = 5.05$. After adding the pure drug to the fixed concentration of the preanalyzed pharmaceutical formulations.

Recovery studies. The reliability and accuracy of the proposed methods were further ascertained through recovery studies using the standard addition method by adding different amounts of the standard drug to the preanalyzed dosage forms so that the cumulative amount after adding the drug did not exceed their linearity range. The results of the recovery study are compiled in Table 5. Comparisons of the proposed methods with the existing methods are shown in Table 6.

TABLE 5. Results of Accuracy Assessment by the Recovery Study

Method	Formulation	Amount of drug taken in tab, µg/mL	Amount of pure drug added, µg/mL	Total found[*], µg/mL	% Recovered ± % RSD
A	Staxyn 10 mg	5.0	1.0	5.99	99.8±0.99
		5.0	7.0	12.01	100.08±1.99
		5.0	13	17.99	99.994±0.97
B	Staxyn 10 mg	8	4	12.1	100.8±0.008
		8	10	17.97	99.8±0.007
		8	16	23.94	99.75±0.51
C	Staxyn 10 mg	10	20	30.01	100.03±0.71
		10	26	35.95	99.86±0.31
		10	32	41.99	99.97±0.23

[*]Mean value of five determinations.

TABLE 6. Comparison of the Proposed Methods with the Existing Methods

Reagents	Methodology	Beer's law limit, μg/mL	Remarks	Reference
Cerium (IV) ammonium sulphate, H_2SO_4 orange G (OG), rhodamine B (RB), methylene blue (MB) and methyle orange (MO)	Unbleached color of OG, RB, MB & MO were measured at 478, 550, 664, and 510 nm	1.0–8.0 $\varepsilon = 4.587\times10^3$ L/mol · cm 1.0–10 $\varepsilon = 3.420\times10^4$ L/mol · cm 1.0–12 $\varepsilon = 2.170\times10^4$ 1.0–12 $\varepsilon = 4.309\times10^4$ L/mol · cm	Narrow linear range, sensitive, and requires acidic medium	Ragaa El Shiekh, et al., 2016 [18]
Potassium iodide and potassium iodate	Yellow-colored complex was measured at 285, 351 nm	0.2–13 $\varepsilon = 68.719\times10^3$ L/mol · cm 0.5–40 $\varepsilon = 26.691\times10^3$ L/mol · cm	Maximum absorption should be more than 400 nm for yellow colored complex	Amir Alhaj Sakur, et al., 2017 [19]
Method A: NBS, KBr, HCl, amaranth Method B: NBS, KBr, HCl methylene blue, Method C: NBS, KBr, HCl indigocarmine or orange G	A: absorbance was measured at 520 nm B: absorbance was measured at 664 nm C: absorbance was measured at 610 or 478 nm	1.0–16 $\varepsilon = 0.971\times 10^4$ L/mol · cm 1.0–12 $\varepsilon = 2.5114\times10^4$ L/mol · cm 1.0–10 $\varepsilon = 2.208\times10^4$ L/mol · cm	Sensitive	Ragaa El Shiekh, et al., 2016 [20]
$KMnO_4$, H_2SO_4, amaranth, indigo carmine and methylene blue	Absorbance of unbleached products were measured at 520, 610, and 664 nm	2.0–12, 2.0–15, 2.0–12 $\varepsilon = 2.0956\times10^4$ $\varepsilon = 1.2138\times10^4$ $\varepsilon = 1.7502\times10^4$ L/mol · cm	Sensitive, same dyes are used in both the methods except the reagent	Ragaa El Shiekh, et al., 2016 [21]
Method A: 3-methyl-2-benzothiazolinone hydrazone hydrochloride, ferric chloride, HCl Method B: 4-aminoantipyrine, potassium periodate, NaOH	A: green-colored chromogen was measured at 625 nm B: red-colored product was measured at 530 nm	4–40 $\varepsilon = 3.774\times10^4$ L/mol · cm 4–60 $\varepsilon = 4.605\times10^4$ L/mol · cm	Reagents are expensive	A.V.V.N.K. Sunil Kumar, et al., 2016 [22]
Method A: $KMnO_4$ in 0.6 M NaOH Method B: bromocresol green, hydrochloric acid, chloroform Method C: acetaldehyde & nitroprusside, sodium hydroxide	A: green-colored manganate ions was measured at 630 nm B: the colored ion-pair complex was measured at 420 nm C: the colored inner molecular complex was measured at 560 nm	10–100 $\varepsilon = 4.299\times10^3$ 5–60 $\varepsilon = 3.755\times10^4$ 2–20 $\varepsilon = 4.079\times10^4$	Requires extraction process, wide linear range	Atkuru Veera Venkata Naga Krishna Sunil Kumar, et al., 2015 [23]
Method A: NBS safranin Method B: NBS and crystal violet Method C: NBS, aniline blue, H_2SO_4	A: the absorbance of pink-colored product was measured at 530 nm B: the absorbance of the violet-colored product was measured at 600 nm C: the absorbance of the blue-colored product was measured at 610 nm	6–36 $\varepsilon = 1.1104\times10^4$ L/mol · cm 6–30 $\varepsilon = 1.0305\times10^4$ L/mol · cm 12–48 $\varepsilon = 6.217\times10^3$ L/mol · cm	Highly sensitive, extraction step, no use of organic solvent and inexpensive instrumental set-up	Present work

Robustness. Robustness was studied by assessing the small variation of the method variables including the concentration of analytical reagents and reaction time on the performance of the proposed methods. In this research, one variable was modified, whereas the others were kept unchanged and the recovery percentage was calculated for each time. It was found that slight variations in these variables did not affect the method significantly. This shows the reliability of the proposed methods during the practice application for the analysis; thus, the recommended spectrophotometric methods are robust.

Conclusions. The present paper exemplified the evaluation of N-bromosuccinimide as a good oxidizing agent in the development of a simple, sensitive, rapid, and economical method with a high degree of precision, as well as being a reliable spectrophotometric method for the determination of Vardenafil in both pure and pharmaceutical dosage forms. The developed methods are more superior in terms of simplicity and sensitivity than the previously reported methods. The methods do not suffer from the unsteadiness of colors, such as bleaching of the dye, and the intact analysis was carried out with in a period of about 25–30 min. Also, the reagents used in the developed methods are inexpensive, readily available, and do not involve major reaction conditions such as extraction or tedious sample preparation steps. Thus, these advantages support the application of the developed methods in pharmaceutical formulations and in quality control laboratories.

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