Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (15):153-165
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Spectrophotometric estimation of vardenafil HCl and tadalafil in pure forms and tablets using cerium(IV) ammonium sulphate

Ragaa El Shiekh[1], Alaa S. Amin[2], Eman M. Hafez[1] and Ayman A. Gouda[1,3]*

[1]Chemistry Department, Faculty of Sciences, Zagazig University, Zagazig, 44519, Egypt
[2]Chemistry Department, Faculty of Sciences, Benha University, Benha, Egypt
[3]Faculty of Public Health and Health Informatics, Umm AL-Qura University, Makkah, Saudi Arabia.

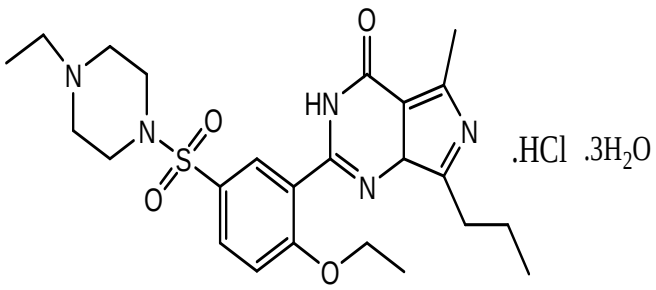
ABSTRACT

A simple, sensitive and accurate spectrophotometric methods have been developed for the determination of two phosphodiesterase type 5-inhibitors; vardenafil HCl (VARD) and tadalafil (TDF) in bulk drugs and pharmaceutical preparations. The methods are based on the oxidation of the studied drugs by a known excess of ceric(IV) ammonium sulphate (CAS) in acid medium followed by determination of unreacted oxidant by adding a fixed amount of orange G (OG), rhodamine B (RB), methylene blue (MB) and methyle orange (MO) dyes followed by measuring the absorbance at 478, 550, 664 and 510 nm, respectively. The experimental conditions affecting the reaction were studied and optimized. The beer's law was obeyed in the concentration ranges of 1.0-8.0, 1.0-10, 1.0-12 and 1.0-12 μg mL^{-1} for VARD using OG, RB, MB and MO methods, respectively and 2.0-12, 1.0-12 and 1.0-15 μg mL^{-1} for TDF using OG, RB and MB methods, respectively with a correlation coefficient ≥ 0.9990. The calculated molar absorptivity values are 4.5874 × 10^4, 3.4207 × 10^4, 2.1705 × 10^4 and 4.3091 × 10^4 L mol^{-1} cm^{-1} for VARD using OG, RB, MB and MO methods, respectively and 3.3086 × 10^4, 5.2058 × 10^4 and 3.3342 × 10^4 L mol^{-1} cm^{-1} for TDF, using OG, RB and MB methods, respectively. The limits of detection and quantification were reported. Intra-day and inter-day accuracy and precision of the methods have been evaluated. No interference was observed from the additives and the applicability of the method was tested by analyzing the pharmaceutical preparations containing the investigated drugs. The methods were successfully applied to the assay of VARD and TDF in tablet preparations and the results were statistically compared with those of the reported methods by applying Student's t-test and F-test. The reliability of the methods was further ascertained by performing recovery studies using the standard addition method.

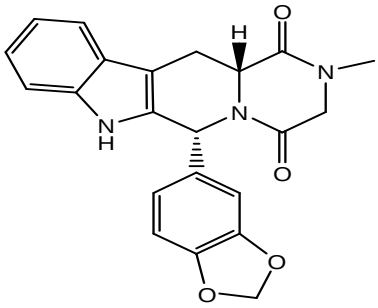
Keywords: Spectrophotometry; Vardenafil HCl; Tadalafil; Ceric(IV); Tablets.

INTRODUCTION

Vardenafil hydrochloride (VARD) is designated chemically as piperazine, 1-[[3-(1,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-f] [1,2,4]triazin-2-yl)-4-ethoxy-phenyl] sulfonyl]-4-ethyl-, monohydrochloride and tadalafil (TDF) is designated chemically as (6*R*-trans)-6-(1,3-benzodioxol-5-yl)- 2,3,6,7,12,12a-hexahydro-2-methyl-pyrazino [1', 2':1,6] pyrido[3,4-*b*]indole-1,4-dione (Figure 1). VARD and TDF are widely used as a selective phosphodiesterase type 5- inhibitor (PDE5) in the management of erectile dysfunction [1,2]. Extensive literature survey revealed that the determination of VARD and TDF in pure and dosage forms are not official in any of the pharmacopoeias and therefore, require much more investigation.



Vardenafil hydrochloride (VARD)



Tadalafil (TDF)

Figure 1. The chemical structure of vardenafil hydrochloride and tadalafil

Few reports for the determination of VARD in pure, tablet dosage forms and biological fluids have been developed with the help of a variety of analytical tools including high performance liquid chromatography [HPLC] [3-12], gas chromatography [13,14], capillary electrophoresis [15,16], electrochemical methods [17,18] and atomic emission spectrometry [19-21]. Several analytical methods have been reported for the estimation of TDF in biological fluids or pharmaceutical dosage forms include HPLC [22-34], liquid chromatography-tandem mass spectrometry with electrospray ionization [35–37], micellar electrokinetic capillary chromatography [38] and atomic emission spectrometry [20,21].

Table 1. Comparison between the report spectrophotometric method for determination of VARD and TDF

Method	Wavelength (nm)	Beer's law (µg mL^{-1})	Molar absorptivity (L $mol^{-1}cm^{-1}$)	Detection limit (µg mL^{-1})	Remarks	References
VARD						
3-methyl-2-benzothiazolinone hydrazone hydrochloride/$FeCl_3$	625	4.0-40	NA	0.044	Less sensitive, less stable species measured	(39)
4-aminoantipyrine / potassium periodate	530	4.0-60	NA	0.035		
BCG	418	2.0-14	2.471×10^4	0.56	Required close pH control and involved extraction steps organic solvent is used	(40)
BCP	410	2.0-20	1.302×10^4	0.49		
BTB	417	1.0-12	4.594×10^4	0.27		
BPB	417	2.0-14	3.284×10^4	0.53		
MO	429	1.0-20	2.48×10^4	0.26		
Ce(IV) /(a) OG	478	1.0-8.0	4.5874×10^4	0.24	Highly sensitive and selective, no heating or extraction step, Inexpensive instrumental setup, use of ecofriendly chemicals, and aqueous system	Present work
(b) RB	550	1.0-10	3.4207×10^4	0.26		
(c) MB	664	1.0-12	2.1705×10^4	0.21		
(d) MO	510	1.0-12	4.3091×10^4	0.28		
TDF						
Ce(IV)/ methyl orange	507	18-60	1.0464×10^4	10.5	Less sensitive	(45)
N-bromosuccinamide/ indigo carmine	610	10-55	1.4922×10^4	5.3		
Ce(IV)/ Indigo carmine	610	11-50	0.8119×10^3	3.5	Less sensitive	(46)
Ce(IV)/ methylene blue	600	10-55	0.8367×10^3	2.3		
Bromocresol purple (BCP)	410	2.0-16	1.332×10^4	0.092	Less sensitive, involves pH control, extraction step	(47)
Methyl orange (MO)	425	2.0-20	1.033×10^4	0.11		
Bromothymol blue (BTB)	420	10-50	NA	2.23	Less sensitive, involves pH control, extraction step	(48)
Bromocresol green (BCG)	415	10-50	NA	2.36		
Isatin	665	2.0-10	7.70×10^3	NA	Less sensitive, use conc. H_2SO_4	(49)
Xanthydrol	640	4.0-20	2.59×10^4	NA		
3-methyl-2-benzothiazoline hydrazone (MBTH)	676	2.0-12	NA	0.0157	Heating required	(50)
Ce(IV) /(a) OG	478	2.0-12	3.3086×10^4	0.54	Highly sensitive and selective, no heating or extraction step, Inexpensive instrumental setup, use of ecofriendly chemicals, and aqueous system	Present work
(b) RB	550	1.0-10	5.2058×10^4	0.23		
(c) MB	664	1.0-15	3.3342×10^4	0.27		

NA: not available.

All the above methods developed for the quantification of VARD and TDF employed complex analytical instruments for their estimation mainly in bulk drug powders, tablet dosage forms and biological fluids. However, most of these methods are complex, require expensive experimental setup and skilled personnel, suffer from time-consuming procedures, and are inaccessible to many laboratories in developing and under developed nations. In

contrast, visible spectrophotometry is considered as the most convenient analytical technique in most quality control and clinical laboratories, hospitals and pharmaceutical industries for the assay of different classes of drugs in pure, pharmaceutical formulations and biological samples, due to its simplicity and reasonable sensitivity with significant economic advantages.

To the best of our knowledge, there are some methods have been reported for the quantification of VARD and TDF in commercial dosage forms using a spectrophotometric technique (38-50) (Table 1). However, these previously reported methods suffer from one or the other disadvantage such as poor sensitivity, depending on critical experimental variables, few methods require a rigid pH control and tedious and time-consuming liquid–liquid extraction step; some other methods have a relatively narrow dynamic linear range, involve a heating step, and/or use of expensive reagent or large amounts of organic solvents. For these reasons, it was worthwhile to develop a new, simple, cost effective and selective spectrophotometric method for the determination of VARD and TDF their pharmaceutical dosage forms.

Orange G (OG), rhodamine B (RB), methylene blue (MB) and methyle orange (MO) dyes are well known for their high absorptivity and have been utilized for estimation of excess oxidant. The present work aims to develop a simple, rapid, sensitive, accurate, precise and validated spectrophotometric method for the estimation of VARD and TDF in pure and dosage forms. The method is based on the oxidation of the investigated drugs with slight excess of CAS in acidic medium. The unconsumed of CAS is then estimated by adding a fixed amount of OG, RB, MB and MO dyes to form colored species which absorbs maximally at 478, 550, 664 and 510 nm, respectively.

MATERIALS AND METHODS

Apparatus

All absorption spectra were made using Varian UV–Vis spectrophotometer (Cary 100 Conc., Australia) equipped with 10 mm quartz cell was used for absorbance measurements. This spectrophotometer has a wavelength accuracy of ±0.2 nm with a scanning speed of 200 nm/min and a bandwidth of 2.0 nm in the wavelength range of 200–900 nm.

Materials and reagents

All chemicals, solvents and reagents used in this work were of analytical reagent or pharmaceutical grade and all solutions were prepared fresh daily. Bidistilled water was used throughout the investigation.

Reference standard of pure drugs

Pharmaceutical grade VARD and TDF working standard was kindly supplied by their respective manufactures in Egypt, without any conflicts of interests in our submitted paper.

Pharmaceutical formulations

The following tablets were purchased from local commercial markets. Levitra tablets are labeled to contain 10 mg VARD per tablet (Bayer HealthCare Pharmaceuticals, Germany). Powerecta tablets are labeled to contain 20 mg VARD per tablet (Eva Pharma Company Giza, Egypt). Verdenodeb tablets are labeled to contain 20 mg VARD per tablet (Debeiky Pharmaceutical, Cairo, Egypt). Cialis® tablets, labeled to contain 20 mg TDF per tablet (Eli Lilly, Australia). Snafi® tablets, labeled to contain 20 mg TDF per tablet (Saudi Pharmaceutical Industries & Medical Appliances Corporation (SPIMACO), Al-Qassim, Saudi Arabia.

Standard solutions

A stock standard solution (100 µg mL^{-1}) of VARD and (200 µg mL^{-1}) TDF was prepared by dissolving 10 and 20 mg of pure VARD and TDF, respectively in bidistilled water and methanol, respectively further diluted to 100 mL with the same solvent in a 100 mL measuring flask. The standard solutions were found stable for at least one week without alteration when kept in an amber colored bottle and stored in a refrigerator when not in use.

Reagents

Cerium(IV) ammonium sulphate (5.0×10^{-3} mol L^{-1})

A stock solution of 5.0×10^{-3} mol L^{-1} cerium(IV) ammonium sulphate (CAS) (E-Merk, Darmstadt, Germany) was freshly prepared by dissolving 316.2 mg CAS in the least amount of H_2SO_4 (2.0 mol L^{-1}) then completed to the mark in a 100 mL calibrated flask with the same acid and kept in a dark bottle and a refrigerator when not in use.

Sulfuric acid (H_2SO_4) (2.0 mol L^{-1})

A stock solution of 2.0 mol L^{-1} H_2SO_4 was prepared by adding 10.8 mL of concentrated acid (Merck, Darmstadt, Germany, 98%, Sp. Gr. 1.84) to bidistilled water, cooled to room temperature, transfer to 100 mL with measuring flask, diluted to the mark and standardized as recorded (51).

Dyes (1000 µg mL^{-1})

A stock solutions (1000 µg mL^{-1}) orange G (OG), rhodamine B (RB), methylene blue (MB) and methyle orange (MO) were first prepared by dissolving accurately weighed 112 mg of each dye (Sigma-aldrish, 90 % dye content) in bidistilled water and diluting to volume in a 100 mL calibrated flask. The solution was then diluted 5.0-fold for OG to get the working concentration of 200 µg mL^{-1} or diluted 10-fold for RB, MB and MO to get the working concentration of 100 µg mL^{-1}.

Recommended general procedures

For VARD

Different aliquots (0.0-0.8 mL), (0.1-1.0 mL), (0.1-1.2 mL) and (0.1-1.2 mL) of a standard 100 µg mL^{-1} VARD solution using OG, RB, MB and MO methods, respectively, were transferred into a series of 10 mL calibrated flasks followed by adding 1.0 mL of 2.0 mol L^{-1} H_2SO_4 and 1.0 mL of (5.0×10^{-3} mol L^{-1}) CAS solution. The flasks were stoppered and the contents were mixed well and the flasks were kept aside for 5.0 min with occasional shaking. Finally, 1.0 mL of (200 µg mL^{-1}) OG and (100 µg mL^{-1}) RB, MB or MO dye solution was added to each flask and mixed well, and then the volume was diluted to the mark with bidistilled water. The decrease in color intensity of dyes were measured after 5.0 min against reagent blank solution treated similarly omitting the drug, at their corresponding λ_{max} 478, 550, 664 or 510 nm, respectively. The concentration of unknown was determined in each case from calibration graph which obtained by plotting the concentration of VARD against absorbance.

For TDF

Different aliquots (0.2-1.2 mL), (0.1-1.0 mL) and (0.1-1.5 mL) of a standard 100 µg mL^{-1} TDF solution using OG, RB and MB methods, respectively, were transferred into a series of 10 mL calibrated flasks followed by adding 1.0 mL of 2.0 mol L^{-1} H_2SO_4 and 1.0 mL of (5.0×10^{-3} mol L^{-1}) CAS solution. The flasks were stoppered and the contents were mixed well and the flasks were kept aside for 5.0 min with occasional shaking. Finally, 1.2 mL of OG (200 µg mL^{-1}) and RB or MB (100 µg mL^{-1}) dye solution was added to each flask and mixed well, and then the volume was diluted to the mark with bidistilled water. The decrease in color intensity of dyes were measured after 5.0 min against reagent blank solution treated similarly omitting the drug, at their corresponding λ_{max} 478, 550 or 664 nm, respectively. The concentration of unknown was determined in each case from calibration graph which obtained by plotting the concentration of TDF against absorbance.

Procedure for pharmaceutical formulations (tablets)

The contents of ten tablets of each drug were accurately weighed and ground into a fine powder. An accurate weight of the powdered tablets equivalent to 20 mg VARD was dissolved in bidistilled water or 20 mg TDF was dissolved in methanol with shaking for 5.0 min and filtered using a Whatman No. 42 filter paper. The filtrate was diluted to the mark with bidistilled water for VARD or methanol for TDF in a 100 mL measuring flask to give and 200 µg mL^{-1} stock solution of VARD or TDF for analysis by the proposed methods. Determine the nominal content of the tablets using the corresponding regression equation of the appropriate calibration graph.

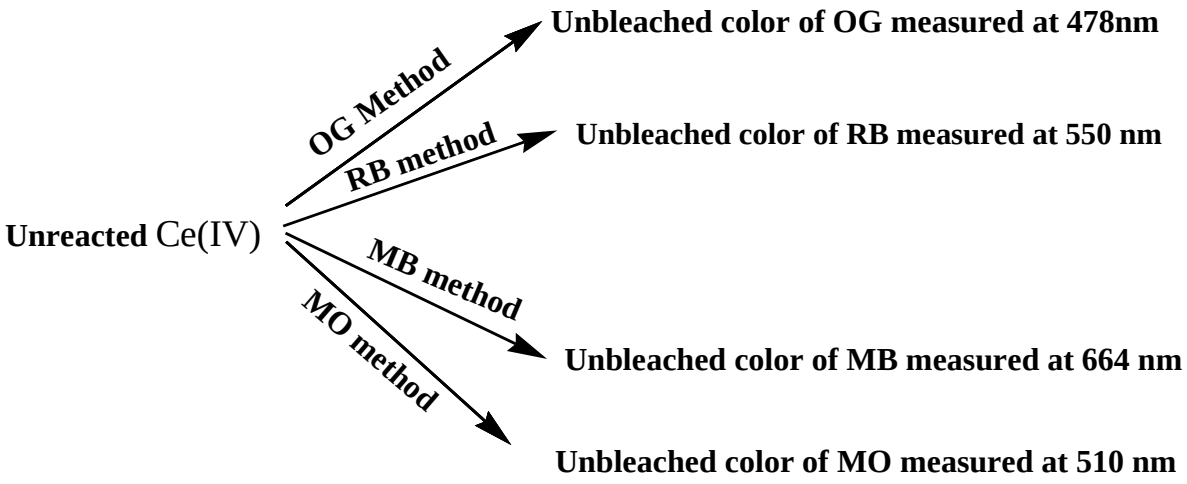
RESULTS AND DISCUSSION

Absorption spectra

Cerium(IV) ammonium sulphate, because of its high oxidation potential and excellent solution stability, has been widely used as an effective analytical reagent in spectrophotometric methods for the determination of many pharmaceutical compounds (52-56). The proposed spectrophotometric method for the determination of VARD and TDF is indirect and involves two steps namely:

1. Oxidation of the studied drugs with a known excess of CAS in acidic medium at room temperature (25 ±2 °C).
2. Determination of the residual CAS by reacting it with a fixed amount of OG, RB, MB or MO dyes and measuring the increase in absorbance at λ_{max} 478, 550, 664 or 510 nm, respectively (Scheme 1).

$$\text{Drug} + \text{Known excess of Ce(IV)} \xrightarrow{H^+} \text{Oxidation product of drug} + \text{Ce(III)} + \text{Unreacted Ce(IV)}$$



Scheme 1. The suggested reaction pathway for the proposed spectrophotometric methods using CAS and dyes

Optimization of the reaction conditions

The optimum conditions for the assay procedures and color development for each method have been established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species.

Effect of acid type and concentration

In order to investigate the effect of acid concentration, different types of acids were examined (HCl, H_2SO_4, H_3PO_4, HNO_3 and CH_3COOH) to achieve maximum yield of redox reactions. The results indicated that the sulphuric acid (H_2SO_4) (2.0 mol L^{-1}) was the most suitable acid with CAS as oxidant. Moreover, different volumes (0.2–3.0 mL) of 2.0 mol L^{-1} H_2SO_4 were tested and found to be a constant absorbance was obtained with 0.5–1.5 mL of H_2SO_4 (2.0 mol L^{-1}), so 1.0 mL of H_2SO_4 (2.0 mol L^{-1}) was the optimum volume for subsequent studies for both drugs (Figure 2).

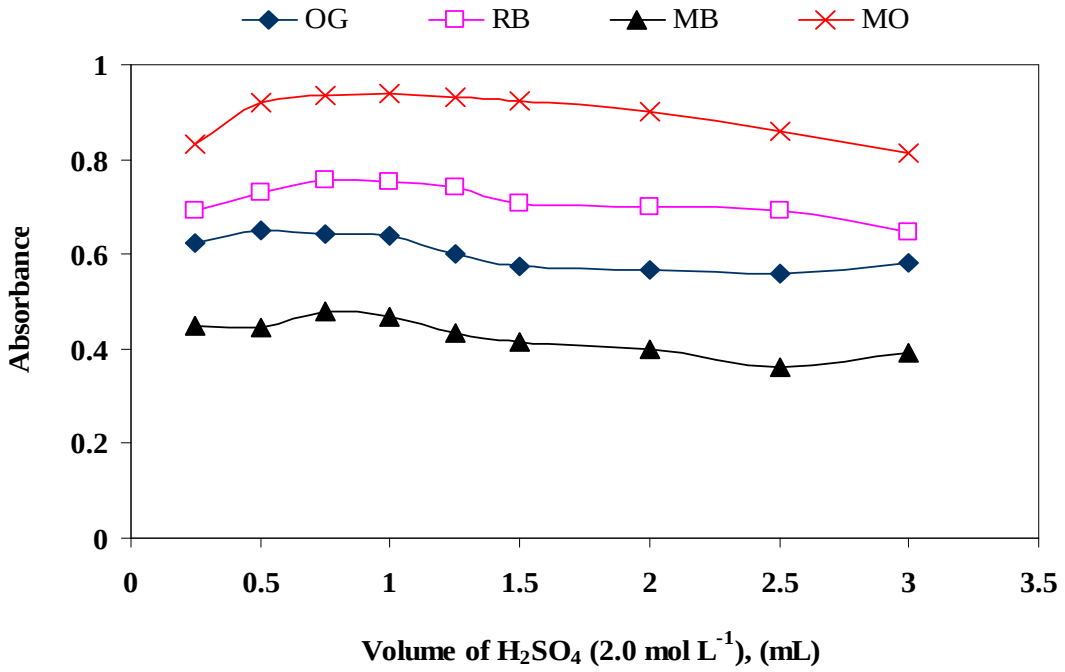


Figure 2. Effect of volume of H_2SO_4 (2.0 mol L^{-1}) on the absorbance of 8.0 µg mL^{-1} VARD with CAS (5.0 x 10^{-3} mol L^{-1}) and (200 µg mL^{-1}) OG or (100 µg mL^{-1}) RB, MB and MO dyes

Effect of CAS concentration

The influence of the concentration of CAS on the absorbance of the colored products was investigated using different volumes of 5.0 × 10^{-3} mol L^{-1} CAS solution from (0.25-3.0 mL). The results indicate that the maximum and constant absorbance was obtained using 1.0 mL of 5.0 × 10^{-3} mol L^{-1} CAS solution and the color intensity decreased

above the upper limits. Therefore, 1.0 mL of 5.0 × 10^{-3} mol L^{-1} CAS was taken as the optimum concentration for all measurements (Figure 3a, 3b).

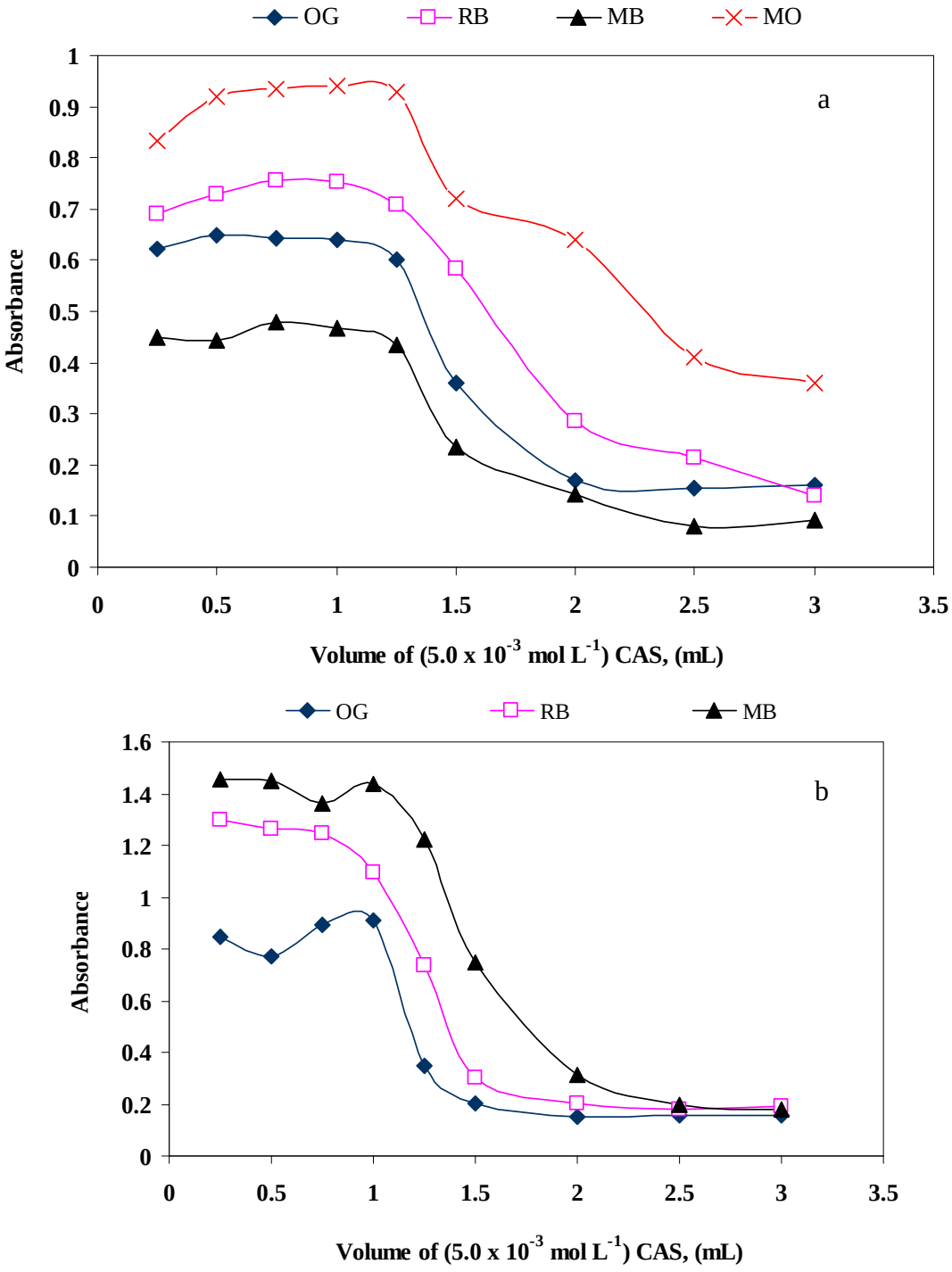


Figure 3. Effect of volume of CAS (5.0 x 10^{-3} mol L^{-1}) on the reaction product of (a) VARD (8.0 µg mL^{-1}) and (b) TDF (10 µg mL^{-1}) with CAS and dyes in H_2SO_4 medium

Effect of dye concentration

The effect of dye concentration on the intensity of the color developed was carried out to obtain the optimum concentration of dyes that produces the maximum and reproducible color intensity by reducing the residual of CAS. The effect dye concentration was studied using different volumes (0.25–3.0 mL) of the studied dyes OG (200 µg mL^{-1}) and RB, MB and MO (100 µg mL^{-1}). It was observed that maximum color intensity of the oxidation products was achieved with 1.0 mL of OG, RB, MB and MO dye solution in case of VARD. Whereas, It was found that

maximum color intensity of the oxidation products was achieved with 1.2 mL of OG, RB and MB dye solutions, respectively for TDF (Figure 4a and 4b). The color was found to be stable up to 24 h.

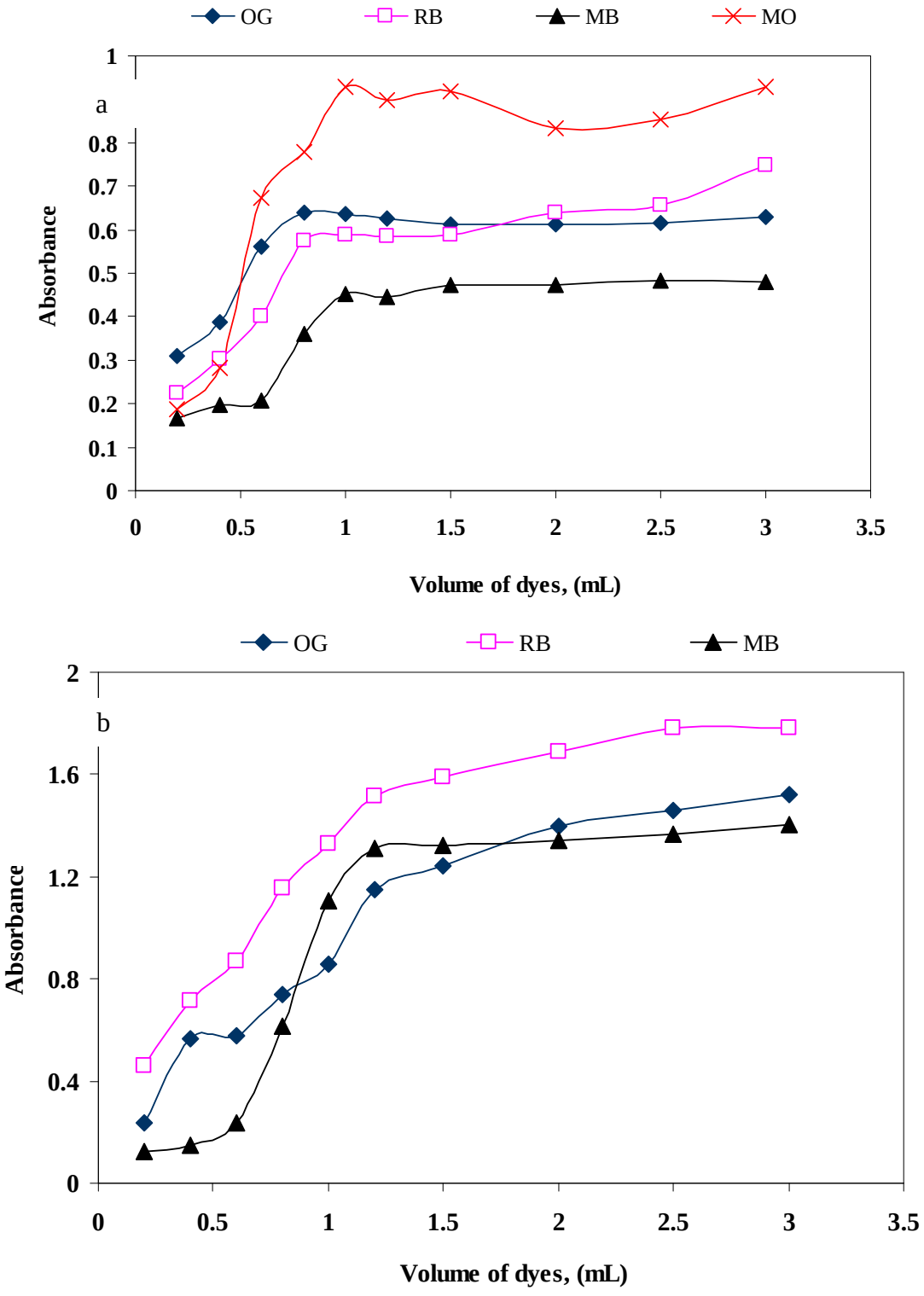


Figure 4. Effect of volume of dyes on the reaction product of (a) VARD (8.0 µg mL^{-1}) and (b) TDF (10 µg mL^{-1}) with CAS and dyes in H_2SO_4 medium

Effect of temperature and mixing time

The effect of temperature was studied by heating a series of sample and blank solutions at different temperatures ranging from 20 to 60 °C in water bath. It was found that raising the temperature does not accelerate the oxidation process and does not give reproducible results, so maximum color intensity was obtained at room temperature (25±2 °C). The effect of mixing time required completing oxidation of the studied drugs and for reducing the excess

oxidant was studied by measuring the absorbance of sample solution against blank solution prepared similarly at various time intervals 2.0–20 min. It was found that the contact times gave constant and reproducible absorbance values at 5.0 min for both drugs. After oxidation process, 5.0 min standing time was found necessary for the complete bleaching of the dye color by the residual CAS for both drugs and the absorbance of the unreacted dye was stable for at least 24 h, thereafter.

Effect of sequence of addition

After optimizing all other experimental variables, further experiments were performed to ascertain the influence of sequence of addition of reactants on the color development by measuring the absorbance. The optimum sequence of addition was drug–H_2SO_4–CAS–dye. Other sequences gave lower absorbance values under the same experimental conditions.

Method validation

The proposed methods have been validated for linearity, sensitivity, precision, accuracy, selectivity and recovery.

Linearity and sensitivity

Under the optimum conditions a linear correlation was found between absorbance at λ_{max} and the concentration of VARD and TDF in the ranges of 1.0-8.0, 1.0-10, 1.0-12 and 1.0-12 µg mL^{-1} for VARD using OG, RB, MB and MO methods, respectively and 2.0-12, 1.0-12 and 1.0-15 µg mL^{-1} for TDF using OG, RB and MB methods, respectively. The calibration graph is described by the equation:

$$A = a + b\,C \qquad (1)$$

Where A= absorbance, a= intercept, b= slope and C= concentration in µg mL^{-1}, obtained by the method of least squares. Correlation coefficient, intercept and slope of the calibration data are summarized in Table 2. For accurate determination, Ringbom concentration range (57) was calculated by plotting log concentration of drug in µg mL^{-1} against transmittance % from which the linear portion of the curve gives an accurate range of microdetermination of VARD and TDF and represented in Table 2. Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, as well as the limits of detection and quantification, were calculated as per the current ICH guidelines (58) and illustrated in Table 2. The high molar absorptivity and lower Sandell's sensitivity values reflect the good and high sensitivity of the proposed methods. The validity of the proposed methods was evaluated by statistical analysis (58) between the results achieved from the proposed methods and that of the reported method. Regarding the calculated Student's *t*-test and variance ratio *F*-test (Table 2), there is no significant difference between the proposed and reported methods (40, 47) regarding accuracy and precision.

Table 2. Analytical and regression parameters of proposed oxidation spectrophotometric methods for determination of VARD and TDF

Parameters	VARD				TDF		
	OG	**RB**	**MB**	**MO**	**OG**	**RB**	**MB**
Beer's law limits, µg mL^{-1}	1.0-8.0	1.0-10	1.0-12	1.0-12	2.0-12	1.0-10	1.0-15
Ringboom limits, µg mL^{-1}	3.0-6.0	3.0-8.0	3.0-10	3.0-10	4.0-10	3.0-8.0	3.0-12
Molar absorptivity, x 10^4 ($L\ mol^{-1}\ cm^{-1}$)	4.5874	3.4207	2.1705	4.3091	3.3086	5.2058	3.3342
Sandell sensitivity, ng cm^{-2}	12.24	16.42	25.87	13.03	11.77	7.48	11.68
Regression equation[a]							
Intercept (a)	0.0019	0.0062	-0.0021	-0.0019	-0.0048	-0.003	-0.0048
SD of intercept (S_a)	0.012	0.018	0.023	0.028	0.008	0.007	0.014
Slope (b)	0.0793	0.0564	0.0386	0.0757	0.086	0.1366	0.0967
SD of slope (S_b)	0.027	0.009	0.017	0.015	0.025	0.029	0.031
Correlation coefficient, (r)	0.9993	0.9990	0.9993	0.9993	0.9997	0.9994	0.9995
Mean ± SD	99.49± 0.98	100.21± 1.12	99.27± 1.31	99.31± 1.04	99.59± 1.10	99.98± 1.20	99.43± 1.33
RSD%	0.99	1.12	1.32	1.04	1.10	1.20	1.34
RE%	1.03	1.17	1.39	1.10	1.16	1.26	1.40
Limit of detection, µg mL^{-1}	0.24	0.26	0.21	0.28	0.54	0.23	0.27
Limit of quantification, µg mL^{-1}	0.80	0.87	0.70	0.93	1.80	0.77	0.90
Calculated *t*-value[b]	1.46	0.09	1.51	1.73	1.11	0.36	1.0
Calculated *F*-value[b]	2.27	2.97	4.06	2.56	1.38	1.16	1.06

[a] *A = a + bC, where C is the concentration in µg mL^{-1}, A is the absorbance units, a is the intercept, b is the slope.*

[b] *The theoretical values of t and F are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p= 0.05).*

The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulas (58, 59):

$$LOD=3.3\sigma/s \quad \text{and} \quad LOQ=10\sigma/s \qquad (2)$$

Where σ is the standard deviation of five reagent blank determinations, and s is the slope of the calibration curve.

Accuracy and precision

In order to evaluate the precision of the proposed methods, solutions containing three different concentrations of VARD and TDF were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Tables 3 and 4. Lower values of the relative standard deviation (R.S.D%) and percentage relative error (R.E%) indicate the precision and accuracy of the proposed methods. The percentage relative error is calculated using the following equation:

$$\% \, R.E. = \left[\frac{found - taken}{taken} \right] \times 100 \qquad (3)$$

The assay procedure was repeated six times, and percentage relative standard deviation (R.S.D%) values were obtained within the same day to evaluate repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision).

For the same concentrations of drugs inter- and intra-day accuracy of the methods was also evaluated. The percentage recovery values with respect to found concentrations of each drug were evaluated to ascertain the accuracy of the methods. The recovery values close to 100% as compiled in Tables 3 and 4 shows that the proposed methods are very accurate.

Table 3. Results of intra-day and inter-day accuracy and precision study for VARD obtained by the proposed methods

Method	Taken (µg mL^{-1})	Intra-day				Inter-day			
		Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]
OG	2.0	99.50	0.42	-0.50	1.99 ± 0.009	99.10	0.47	-0.90	1.982 ± 0.01
	4.0	99.10	0.69	-0.90	3.964 ± 0.029	99.60	0.82	-0.40	3.984 ± 0.034
	6.0	99.40	0.87	-0.60	5.964 ± 0.054	100.30	1.15	-1.0	6.018 ± 0.073
RB	2.0	99.30	0.57	-0.70	1.986± 0.012	99.80	0.63	-0.20	1.996 ± 0.013
	4.0	99.90	0.80	-0.10	3.996 ± 0.034	99.00	0.96	-0.10	3.960 ± 0.04
	8.0	99.20	1.10	-0.80	7.936 ± 0.092	100.60	1.30	0.60	8.048 ± 0.11
MB	2.0	100.40	0.60	0.40	2.008 ± 0.013	99.50	0.53	-0.50	1.990 ± 0.011
	6.0	99.70	0.93	-0.30	5.982 ± 0.058	99.20	0.72	-0.80	5.952 ± 0.045
	10	99.10	1.31	-0.90	9.910 ± 0.136	100.70	0.96	0.70	10.07 ± 0.101
MO	2.0	99.00	0.67	-1.00	1.980 ± 0.014	99.60	0.64	-0.40	1.992 ± 0.013
	6.0	100.20	0.92	0.20	6.012± 0.058	99.10	0.76	-0.80	5.946 ± 0.047
	10	100.50	1.25	0.50	10.05 ± 0.132	99.60	1.40	-0.40	9.960 ± 0.146

[a] *RSD%, percentage relative standard deviation; RE%, percentage relative error.*
[b] *Mean ± standard error.*

Robustness and ruggedness

Robustness was examined by evaluating the influence of small variation of method variables, including concentration of analytical reagents and reaction time on the performance of the proposed methods. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. The analysis was performed with altered conditions by taking three different concentrations of drugs and it was found that small variation of method variables did not significantly affect the procedures as shown by the RSD values in the ranges of 0.70-2.50% and 0.65-2.35% for VARD and TDF, respectively. This provided an indication for the reliability of the proposed methods during its routine application for the analysis of VARD and TDF and so the proposed spectrophotometric methods are considered robust. Ruggedness was expressed as the RSD and was also tested by applying the proposed methods to the assay of VARD and TDF using the same operational conditions but using three different instruments as well as three different anaysts. The inter-analysts RSD were in the ranges 0.90-2.40% and 0.75-2.40% for VARD and TDF, respectively, whereas the inter-instruments RSD ranged

from 0.70-2.25% and 0.80-2.40% for VARD and TDF, respectively suggesting that the developed methods were rugged. The results are shown in Table 5.

Table 4. Results of intra-day and inter-day accuracy and precision study for TDF obtained by the proposed methods

Method	Taken (µg mL^{-1})	Intra-day				Inter-day			
		Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]
OG	4.0	99.30	0.70	-0.70	3.972± 0.029	100.10	0.60	0.10	4.004 ± 0.025
	8.0	99.00	0.65	-1.00	7.920 ± 0.054	99.50	0.88	-0.50	7.960 ± 0.074
	12	99.70	0.89	-0.30	11.964 ± 0.112	99.30	1.32	-0.70	11.916 ± 0.165
RB	2.0	99.10	0.63	-0.90	1.982± 0.013	99.60	0.45	-0.40	1.992 ± 0.009
	4.0	99.20	0.85	-0.80	3.968 ± 0.035	99.00	0.76	-1.00	3.96 ± 0.032
	8.0	100.40	1.30	0.40	8.032 ± 0.11	99.40	1.10	-0.20	7.952 ± 0.092
MB	4.0	99.30	0.70	-0.70	3.972 ± 0.029	100.50	0.68	0.50	4.02 ± 0.029
	8.0	100.70	0.94	0.70	8.056 ± 0.079	98.70	0.95	-0.80	7.896 ± 0.079
	12	99.00	1.45	-0.60	11.88 ± 0.181	100.30	1.20	0.30	12.024 ± 0.151

[a] RSD%, percentage relative standard deviation; RE%, percentage relative error.
[b] Mean ± standard error.

Table 5. Results of method robustness and ruggedness (all values in RSD%) studies for VARD and TDF.

Methods	Nominal amount concentration (µg mL^{-1})	RSD%			
		Robustness		Ruggedness	
		Variable alerted [a]			
		Reagent volume (n=3)	Reaction time (n=3)	Different analysts (n=3)	Different instruments (n=3)
		VARD			
OG	2.0	1.15	0.70	0.90	0.85
	4.0	1.56	1.40	1.60	1.40
	6.0	1.90	2.20	2.10	2.40
RB	2.0	1.20	0.80	1.05	1.10
	6.0	1.70	1.40	1.45	1.50
	8.0	2.00	2.30	2.10	2.25
MB	2.0	0.80	1.15	1.20	1.30
	6.0	1.60	1.70	1.65	1.50
	10	2.40	2.50	2.40	2.10
MO	2.0	0.90	0.70	1.20	0.75
	6.0	1.70	1.30	1.65	1.40
	10	2.30	1.80	2.40	2.20
		TDF			
OG	4.0	0.70	1.08	0.80	0.90
	8.0	1.10	1.65	1.25	1.40
	12	1.70	2.35	1.90	2.10
RB	2.0	0.90	0.87	0.70	1.30
	4.0	1.52	1.35	1.20	1.90
	8.0	2.05	1.80	1.95	2.40
MB	4.0	0.84	0.65	1.10	0.80
	8.0	1.40	1.20	1.75	1.50
	12	2.30	1.80	2.25	2.20

[a] Volume of (2.0 mol L^{-1}) H_2SO_4 is (1.0±0.2 mL) and reaction time is (5.0±2.0 min) (after adding CAS were used.

Recovery studies

To ascertain the accuracy, reliability and validity of the proposed methods, recovery experiment was performed through standard addition technique. This study was performed by spiking three different levels of pure drugs (50, 100 and 150% of the level present in the tablet) to a fixed amount of drugs in tablet powder (pre-analysed) and the total concentration was found by the proposed methods. The determination with each level was repeated three times and the percent recovery of the added standard was calculated from:

$$\textbf{\% Recovery} = \frac{[C_F - C_T]}{C_p} \text{x } 100 \qquad (4)$$

Where C_F is the total concentration of the analyte found, C_T is a concentration of the analyte present in the tablet preparation; C_P is a concentration of analyte (pure drugs) added to tablets preparations. The results of this study presented in Table 6 revealed that the accuracy of the proposed methods was unaffected by the various excipients present in tablets which did not interfere in the assay.

Table 6. Results of recovery experiments by standard addition method for the determination of VARD and TDF in tablets using the proposed methods

Samples	Taken drug in tablet (µg mL^{-1})	Pure drug Added (µg mL^{-1})	OG		RB		MB		MO	
			Total found (µg mL^{-1})	Recovery [a] (%) ± SD	Total found (µg mL^{-1})	Recovery [a] (%) ± SD	Total found (µg mL^{-1})	Recovery [a] (%) ± SD	Total found (µg mL^{-1})	Recovery [a] (%) ± SD
Levitra tablets (10 mg VARD)	3.0	1.5	4.464	99.20±0.30	4.446	98.80±0.40	4.469	99.30±0.37	4.473	99.40±0.47
	3.0	3.0	5.958	99.30±0.61	5.976	99.60±0.75	5.94	99.00±0.50	6.006	100.10±0.73
	3.0	4.5	7.463	99.50±0.89	7.448	99.30±1.20	7.418	98.90±0.90	7.545	100.60±1.25
Powerecta tablets (20 mg VARD)	3.0	1.5	4.536	100.80±0.52	4.478	99.50±0.60	4.455	99.00±0.55	4.487	99.70±0.70
	3.0	3.0	5.94	99.00±0.72	6.018	100.30±0.84	5.946	99.10±0.70	5.952	99.20±0.85
	3.0	4.5	7.478	99.70±0.96	7.418	98.90±0.95	7.53	100.40±1.10	7.463	99.50±1.30
Verdenodeb tablets (20 mg VARD)	3.0	1.5	4.455	99.00±0.50	4.464	99.20±0.55	4.482	99.60±0.49	4.460	99.10±0.51
	3.0	3.0	5.976	99.60±0.73	5.922	98.70±0.92	6.042	100.70±0.67	5.964	99.40±0.86
	3.0	4.5	7.433	99.10±0.90	7.448	99.30±1.40	7.478	99.70±0.90	7.508	100.10±0.96
Cialis® **tablets (20 mg TDF)**	4.0	2.0	6.024	100.40±0.49	5.946	99.10±0.60	5.964	99.40±0.70		
	4.0	4.0	7.96	99.50±0.65	7.984	99.80±0.80	8.056	100.70±0.95		
	4.0	6.0	10.01	100.10±1.03	9.95	99.50±1.05	9.94	99.40±1.10		
Snafi® **tablets (20 mg TDF)**	4.0	2.0	5.94	99.00±0.48	5.958	99.30±0.38	6.012	100.20±0.50		
	4.0	4.0	7.944	99.30±0.71	7.976	99.70±0.74	7.968	99.60±1.05		
	4.0	6.0	9.85	98.50±0.95	9.92	99.20±0.98	9.91	99.10±1.20		

[a] *Average of six determinations.*

Table 7. Results of analysis of tablets by the proposed methods for the determination of VARD and TDF and statistical comparison with the reference methods

Samples	Recovery [a] (%) ± SD				Reported methods
	Proposed Methods				
	OG	RB	MB	MO	
Levitra tablets (10 mg VARD)	99.50 ± 0.47	99.40 ± 0.52	99.70 ± 0.84	100.20 ± 0.78	99.92 ± 0.64 [40]
t-value [b]	1.18	1.41	0.47	0.62	
F-value [b]	1.85	1.51	1.72	1.49	
Powerecta tablets (20 mg VARD)	99.60 ± 0.39	99.86 ± 0.90	99.50 ± 0.76	99.30 ± 0.60	99.90 ± 0.67 [40]
t-value [b]	1.47	0.08	0.88	1.49	
F-value [b]	2.95	1.80	1.29	1.25	
Verdenodeb tablets (20 mg VARD)	99.80 ± 0.98	99.10±0.58	100.10±1.04	99.30±0.60	99.50 ± 0.72 [40]
t-value [b]	0.55	0.97	1.06	0.48	
F-value [b]	1.85	1.54	2.09	1.44	
Cialis® tablets (20 mg TDF)	99.42±0.40	99.90±0.63	100.20±0.35		99.79±0.56 [47]
t-value [b]	1.20	0.29	1.39		
F-value [b]	1.96	1.27	2.56		
Snafi® tablets (20 mg TDF)	99.20±0.46	99.10±0.82	99.25±0.68		99.60±0.51 [47]
t-value [b]	1.30	1.16	0.92		
F-value [b]	1.23	2.59	1.78		

[a] *Average of six determinations.*

[b] *The theoretical values of t and F are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05).*

Application of pharmaceutical formulations (tablets)

The proposed methods were applied to the determination of VARD and TDF in pharmaceutical formulations (tablets). The results in Table 7 showed that the methods are successful for the determination of VARD and TDF and that the excipients in the dosage forms do not interfere. A statistical comparison of the results obtained from the assay of VARD and TDF by the proposed methods and the reported methods[40, 47] for the same batch of material is presented in Table 7. The results agree well with the label claim and also were in agreement with the results obtained by the reported methods (40, 47). When the results were statistically compared with those of the reported methods by applying the Student's t-test for accuracy and F-test for precision, the calculated t-value and F-value at

95% confidence level did not exceed the tabulated values for five degrees of freedom (59). Hence, no significant difference between the proposed methods and the reported methods at the 95 % confidence level with respect to accuracy and precision.

CONCLUSION

A new, useful simple, rapid and cost-effective spectrophotometric methods have been developed for determination of VARD and TDF in bulk drugs and in their tablets using CAS as oxidizing agent and validated as per the current ICH guidelines. The present spectrophotometric methods are characterized by simplicity of operation, high selectivity, comparable sensitivity, low-cost instrument, they do not involve any critical experimental variable and are free from tedious and time-consuming extraction steps and use of organic solvents unlike many of the previous methods reported for VARD and TDF. The assay methods have some additional advantages involve less stringent control of experimental parameters such as the stability of the colored system, accuracy, reproducibility, time of analysis, temperature independence and cheaper chemicals. These advantages encourage the application of the proposed methods in routine quality control analysis of VARD and TDF in pure and dosage forms.

REFERENCES

[1] A.A.M. Abdel-Aziz, Y.A. Asiri, A.S. El-Azab, M.A. Al-Omar, T. Kunieda, *Anal Profiles. Drug. Subst. Excipients*, **2011**, 36, 287.
[2] A.E. Ashour, A.F.M.M. Rahman, M.G. Kassem, *Anal. Profiles. Drug. Subst. Excipients*,**2014**, 39, 515.
[3] H.Y. Aboul-Enein, A. Ghanem, H. Hoenen, *J. Liq. Chromatogr. Relat. Technol.*, **2005**, 28, 593.
[4] P. Zou, S.S. Oh, P. Hou, M. Low, H. Koh, *J. Chromatogr. A.*, **2006**, 1104, 113.
[5] X. Zhu, S. Xiao, B. Chen, F. Zhang, S. Yao, Z. Wan, D. Yang, H. Han, *J. Chromatogr. A*, **2005**, 1066, 89.
[6] Z. Zhang, S. Kang, M. Xu, M. Ma, B. Chen, S. Yao, *Se. Pu.*, **2005**, 23, 358.
[7] D.V. Subba Rao, K.V. Surendranath, P. Radhakrishnanand, M.V. Suryanarayana, P. Raghuram, *Chromatographia*, **2008**, 68, 829.
[8] Bartošová Z, Jirovský D and Horna A, *J Chromatogr A.*, **2011**, 1218, 7996 .
[9] S.T. Lake, P.M. Altman, J. Vaisman, R.S. Addison, *Biomed. Chromatogr.*, **2010**, 24, 846.
[10] G. Manisha, P. Usha, P. Vandana, *Am. J. Pharm Tech Res*, **2013**, 3, 928.
[11] Y. Di, M. Zhao, Y. Nie, F. Wang, J. Lv, *J. Autom. Methods. Manag. Chem.*, **2011**, 1, 6.
[12] K. K. Kumar, C. K. Rao, Y.R.K. Reddy, K.A. Mukkanti, *Am. J. Anal. Chem.*, **2012**, 3, 59.
[13] I. Papoutsis, P. Nikolaou, S. Athanaselis, C. Pistos, C. Maravelias, C. Spiliopoulou, *J. Mass. Spectrom.*, **2011**, 46, 71.
[14] S. Strano-Rossi, L. Anzillotti, X. de la Torre, F. Botrè, *Rapid. Commun. Mass. Spectrom*, **2010**, 24, 1697.
[15] Idris A.M., A.O. Alnajjar, *Acta. Chromatogr.*, **2007**, 19, 97.
[16] J.R. Flores, J.J.B. Nevado, G.C. Penalvo, N.M. Diez, *J. Chromatogr. B.*, **2004**, 811, 231.
[17] B.B. Uslu, S.A. Dogan, Ozkan, H.Y. Aboul-Enein, *Anal. Chim. Acta.*, **2005**, 552 127.
[18] Ghoneim M.M., A.M. Hassanein, N.A. Salahuddin, H.S. El-Desoky, M.N. El fiky, *J. Solid. State. Electrochem.*, **2013**, 17, 891.
[19] S. Khalil, *Mikrochemica. Acta.*, **2007**, 158, 233.
[20] S.K.H. Mohammed, N.M. Shalaby, *J. Pharm. Bio. Sci.*, **2013**, 4, 1037.
[21] S.K.H. Mohammed, S.S. Al zahrani, Y.M. Hussein, A.I. Turkestani, *Anal. Chem. An. Indian. J.*, **2014**, 14, 201.
[22] L, Rabbaa-Khabbaz, R.A. Daoud, *J. Appl. Res.*, **2006**, 6, 170.
[23] W. Gao, Z. Zhang, Z. Li, G. Liang, *J. Chromatogr. Sci.*, **2007**, 45, 540.
[24] C.A. Farthing, D.E. Farthing, S. Koka, T. Larus, I. Fakhry, L. Xi, R.C. Kukreja, D. Sica, T.W. Gehr, *J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci.*, **2010**, 878, 2891.
[25] T.G. Barot, P.K. Patel, *J. AOAC Int.*, **2010**, 93, 516.
[26] M.M. Mehanna, A.M. Motawaa, M.W. Samaha, *J. AOAC Int.*, **2012**, 95, 1064.
[27] E. Gudipati, S.D. Mahaboob, B.R. Nunna, K.V. Ashok, K. Rambabu, *Res. Desk.*, **2012**, 1, 66.
[28] P. Meejung, A. Suyoun, *J. Forensic. Sci.*, **2012** ,57, 637.
[29] S. Alivelu, P. Santhosh, M. Sowmya, C. Sravanthi, M. Nageshwar, *J. Chem. Pharm. Res.*, **2013**, 5, 315.
[30] R.B. Prasanna, R.K. Amarnadh, M.S. Reddy, *Res. Pharm. Biotechnol.*, **2010**, 2, 1.
[31] N. Kannappan, Y. Deepthi, Y. Divya, S. Shashikanth, R. Mannavalan, *Int. J. Chem. Tech. Res.*, **2010**, 2, 329.
[32] P.H. Sonawane, P.S. Panzade, M.A. Kale, *Indian. J. Pharm. Sci*, **2013**, 75, 230.
[33] J.K. Patel, N.K. Patel, *Sci. Pharm*, **2014**, 82, 749.
[34] H.Y. Aboul-Enein, I. Ali , *Talanta*, **2005**, 65 ,276.
[35] N.V. Ramakrishna, K.N. Vishwottam, S. Puran, *J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci.*, **2004**, 809, 243.
[36] S.R. Gratz, C.L. Flurer, K.A. Wolnik, *J. Pharm. Biomed. Anal.*, **2004**, 36 ,525.
[37] K. Jomoorthy, B.R. Challa, *Der. Pharm.. Lett.*, **2012**, 4, 1401.

[38] F.J. Rodriguez, N.J.J. Berzas, P.G. Castenada, D.N. Mora, *J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci*., **2004**, 811, 231.
[39] A.V.V.N.K. Sunil Kumara, T.V. Reddyb, C.B. Sekaranc, *Anal. Bioanal. Chem. Res,* **2016**, 3, 29.
[40] R. El Sheikh, M. Zaky, A.A. Gouda, S. Abo Al Ezz, *J. Chil. Chem. Soc*, **2014**, 59, 2248.
[41] M.M. Abdel-Moety, E.R. Souaya, E.A. Soliman, *J. Pharm. Pharm. Sci*, **2015**, 4, 120.
[42] N.B. Savjiyani, P.B. Patel, *J. Pharm. Res*, **2013**, 3, 3652.
[43] N.R. Ahmed, *Baghdad. Sci. J.*, **2013**, 10, 1005.
[44] M. Yunoos , D.G. Sankar, B.P. Kumar, S. Hameed, *E-J. Chem.*, **2010**, 7, 833.
[45] S. Fraihat, *Discovery*, **2014**, 22, 45.
[46] S. *Fraihat, Int. J. Pharm. Pharm. Sci*, **2014**, 6, 443.
[47] A.A. Kaf, A.A. Gouda, *Chem. Ind. Chem. Engin. Quart*, **2011**, 17, 125.
[48] A.J.J. Nesalin, J.G.C. Babu, V.G. Kumar, T.T. Mani, *E-J. Chem.*, **2009**, 6, 611.
[49] V.N. Lakshmi, D.R. Kumar, S.V.M. Vardhan, C. Rambabu, *Orient. J. Chem*, **2009**, 25, 791.
[50] P.K.D. Anumolu, A. Kavitha, D.V. Durga, S.H. Bindu G. Sunitha, K. Ramakrishna, *Anal. Chem. An. Indian. J*, **2013**, 13, 361.
[51] G.H. Jeffery, J. Bassett, J. Mendham, R.C. Denney, Titrimetric analysis. *In Vogel's a text book of quantitative inorganic analysis*, 5th Ed., ELBS: London, **1989**; p.308.
[52] H.A. Abdellatef, M.M. El-Henawee, H.M. El-Sayed, M.M. Ayad, *Spectrochim Acta A.*, **2006**, 65, 997.
[53] Krebs A, Starczewska B, Puzanowska-Tarasiewicz H, Sledz *J, Anal Sci*, **2006**, 22, 829.
[54] A.M. El-Didamony, E.A.H. Erfan, *Spectrochim. Acta. A.*, **2010**, 75, 1138.
[55] A.M. El-Didamony, E.A.H. Erfan, *J. Chil. Chem. Soc*, **2011**, 56, 875.
[56] A.M. El-Didamony, W.S. Hassan, *J. Chil. Chem. Soc*, **2012**, 57, 1404.
[57] A. Ringbom, *Z. Anal. Chem*., **1939**, 115, 332.
[58] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: *Text and Methodology Q2(R 1), Complementary Guideline on Methodology*, London, November. **2005**.
[59] J.N. Miller, J.C. Miller, "Statistics and chemometrics for analytical chemistry" 5th ed., Prentice Hall, England, **2005**.