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RESEARCH ARTICLE

Utilitization of Charge Transfer Complexation Reaction for the Spectrophotometric Determination of Vardenafil HCl and Yohimbin HCl in Pharmaceutical Formulations

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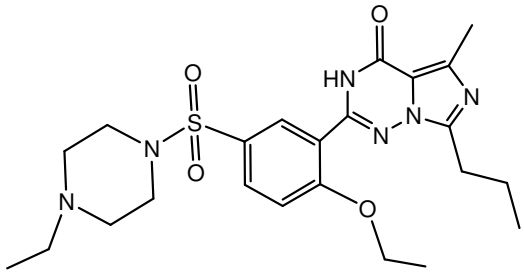
Abstract: A facile, accurate, sensitive and validated spectrophotometric methods for the determination of vardenafil HCl (VARD) and yohimbin HCl (YOHM) in pure and in dosage forms are described. The methods are based on the formation of charge transfer reactions of both drugs as n-electron donor with various π-acceptors: 7,7,8,8-tetracyanoquinodimethane (TCNQ), 2,3-dichloro-5,6 dicyano-*p*-benzoquinone (DDQ) and chloranilic acid (*p*-CLA) to give highly colored complex species. The colored products were quantitated spectrophotometrically at 535, 461 and 840 nm for *p*-CLA, DDQ and TCNQ, respectively for VARD and at 461 and 841 nm using DDQ and TCNQ reagent, respectively for YOHM. The optimization of the reaction conditions such as the type of solvent, reagent concentration and reaction time were investigated. Beer's law is obeyed in the concentration ranges 5.0-200 μg mL^{-1} with good correlation coefficient was ≥0.9995 with a relative standard deviation (R.S.D.) of ≤ 0.70%. The molar absorptivity, Sandell sensitivity, detection and quantification limits were also calculated. The proposed methods were successfully applied for determination of VARD and YOHM in tablets with good accuracy and precision and without interferences from common additives by applying the standard addition technique. Developed methods have been validated statistically for their accuracy, precision, sensitivity, selectivity, robustness and ruggedness as per ICH guidelines and the results compared favourably with those obtained using the reported methods.

Keywords: Vardenafil HCl, Yohimbin HCl, Charge transfer complexes, Spectrophotometry, Pharmaceutical analysis

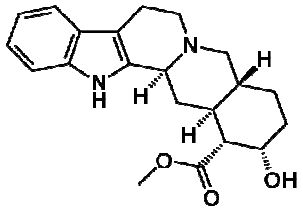
Introduction

Vardenafil HCl (VARD), 1-[[3-(1,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-f][1,2,4] triazin-2-yl)-4-ethoxyphenyl]sulfo-nyl]-4-ethyl- mono hydrochloride (Scheme 1) is used to

treat erectile dysfunction. Vardenafil and other ED drugs inhibit phosphodiesterase type 5 (PDE-5) enzyme, which in turn maintains higher levels of cyclic guanosine monophosphate, which relaxes smooth muscles, promotes penile blood flow and enhances erectile function[1,2]. Yohimbine HCl (YOHM), Methyl 17α-hydroxyyohimban-16α-carboxylate hydrochloride (Scheme 1) is the most important of the complex indole alkaloid types[3]. It is derived from yohimbehe bark which occurs in various tropical trees such as Pausinystalia yohimbe. Pierre, aspidosperma quebrachoblanco schiecht and as a minor alkaloid in some Rawaolfia Species. Yohimbine is an indole alkaloid available commercially as yohimbex R tablet. It's commonly used as an aphrodisiac and in treatment of male impotence. pharmacologically classified as an alpha-2 adrenoreceptor antagonist with some antidopaminergic properties. It can be also used for general purposes such as treatment of orthostatic hypotension, some forms of obesity and diabetic neuropathy[4].



Vardenafil HCl (VARD)



Yohimbine HCl (YOHM)

Scheme 1. The chemical structure of the studied drugs

There is no official method for the determination of VARD in its formulations. Few reports have been published on the determination of Vd, including chromatographic methods[5-12], gas chromatography[13,14], electrokinetic capillary chromatography[15,16], electrochemical methods[17,18] and atomic emission spectrometry[19].

Several methods have been reported for the analysis of YOHM in pure form and pharmaceutical preparations such as spectrophotometric methods[20-25], electrochemical methods using anodic voltammetry[26], potentiometric determination using picrate ion selective electrode[27] and chemiluminometric method[28]. A few chromatographic methods for determination of yohimbine in pharmaceutical preparations and biological samples using different techniques of detection[29-33] were discussed.

All the above methods developed for the quantification of VARD and YOHM employed complex analytical instruments such as mass spectrophotometer for their estimation mainly in dietary supplements and bulk drug powders. Since most of these methods are complicated, expensive, require careful control of conditions and suffer from time-consuming extraction procedures, the use of a simpler, faster, less expensive and all the same sensitive method is desirable. As far as we are aware there is no charge transfer spectrophotometric method for determination and quantitative estimation of VARD and YOHM.

The molecular interactions between electron donors and acceptors are generally associated with the formation of intensely colored charge transfer complexes, which absorb radiation in the visible region[35]. A variety of electron donating compounds have been reported to yield charge-transfer complexes with various π-acceptors[36-39].

The aim of the present study was directed to investigate simple, direct, sensitive, normal cost and precise spectrophotometric methods for simultaneous determination of VARD and

YOHM as a good *n*-electron donor *via* charge transfer complexation with π-acceptors; 7,7,8,8-tetracyanoquinodimethane (TCNQ) 2,3-dichloro-5,6 dicyano-*p*-benzoquinone (DDQ) or *p*- chloranilic acid (*p*-CLA) as chromogenic reagents in pure form and its dosage forms (tablets). The reaction conditions of the methods have been established. In addition, the molar ratio of reactants was determined. No interference was observed in the assay of VARD and YOHM from common excipients in levels found in pharmaceutical formulations. These methods are validated by the statistical data.

Experimental

All absorption spectra were made using double beam Unikon 930 spectrophotometer (Kontron Instruments, Munchen, Germany) with a scanning speed of 200 nm/min and a band width of 2.0 nm, equipped with 10 mm matched quartz cells.

Materials and reagents

All reagents and chemicals used were of analytical or pharmaceutical grade and all solutions were prepared fresh daily.

Standard drug solutions

A stock standard solutions of VARD (Bayer Co., Leverkusen, Germany) and YOHM (Amirya Pharmaceutical Industries, Alexandria, Egypt) were prepared by dissolving 50 mg in 5.0 mL methanol and the volume was diluted to the mark in a 100 mL calibrated flask with acetonitrile to obtain stock solution of 500 µg mL^{-1} of drugs. A stock solution of VARD or YOHM (100 µg mL^{-1}) was prepared from suitable dilution of the stock standard solution and ($5.0x10^{-3}$ M) were also prepared daily. A stock solution of VARD or YOHM ($1.0x10^{-3}$ M) were prepared by dissolving the appropriate weight of drug in 10 mL methanol and the volume was diluted to the mark in a 100 mL calibrated flask with acetonitrile. Such drug solutions are stable for a period of 3.0 days when stored at 4 ºC.

Reagents

7,7,8,8-Tetracyanoquinodimethane (TCNQ), (Aldrich Chem. Co., Milwaukee, USA); ($1.0x10^{-3}$M) solutions in acetonitrile. 2, 3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ), (Merck-Schuchardt, Munich, Germany); ($1.0x10^{-3}$M) solutions in acetonitrile. Chloranilic acid (*p*-CLA), (Fluka, Switzerland) were freshly prepared as ($1.0x10^{-3}$ M) solutions in acetonitrile. The solutions were stable for at least one week at 4 ºC.

General procedures

VARD

Into 10 mL calibrated flasks containing 0.1-2.0 mL and 0.2-4.0 mL of (500 µg mL^{-1}) VARD using *p*-CLA and DDQ methods, respectively and 0.5-4.0 mL of (100 µg mL^{-1}) VARD using TCNQ method, 2.0, 1.5 and 1.0 mL of (1.0×10^{-3}M) *p*-CLA, DDQ and TCNQ, respectively were added. The reaction mixture was heated in a water-bath at 60±2 ºC for 5.0, 10 and 15 min for *p*-CLA, DDQ and TCNQ methods respectively. The mixture was cooled and then diluted to volume up to 10 mL with acetonitrile and the absorbance was measured at 535, 461 and 840 nm for *p*-CLA, DDQ and TCNQ, respectively against a reagent blanks prepared in the same manner.

YOHM

Into 10 mL calibrated flasks containing 0.2-3.2 mL of (500 µg mL^{-1}) YOHM using DDQ method and 0.5-3.0 mL of (100 µg mL^{-1}) YOHM using TCNQ method, 2.0 and 1.0 mL of (1.0×10^{-3}M) DDQ and TCNQ reagent, respectively were added.

The reaction mixture was heated in a water-bath at 60±2 ºC for 10 and 15 min for DDQ and TCNQ, respectively. The mixture was cooled and then diluted to volume up to 10 mL with acetonitrile and the absorbance was measured at 461 and 841 nm using DDQ and TCNQ reagent, respectively, against a reagent blanks prepared in the same manner.

Procedures for pharmaceutical formulations (Tablets)

The contents of twenty tablets (levitra, 10 mg VARD per tablet or Yohimbex, 5.4 mg YOHM per tablet) obtained from a local pharmacy were crushed, then grounded in a mortar to a homogeneous finely powdered, weighed out and the average weight of one tablet was determined for each drug. An accurate weight equivalent to 50 mg VARD or YOHM was transferred into a 100 mL calibrated flask, dissolved in 50 mL methanol (Merk) with shaking for 10 min and filtered through a sintered glass crucible (G_4). The first 5.0 mL portion of the filtrate was rejected and the filtrate was diluted to 100 mL with acetonitrile in a 100 mL measuring flask to give 500 µg mL^{-1} stock solutions. Aliquot of the cited solutions was taken and analyzed as described under the above recommended procedures for construction of calibration curves. For the proposed methods, the content of a tablet was calculated using the corresponding regression equation of the appropriate calibration graph.

Stoichiometric relationship

The Job's method of continuous variation[40] was employed to establish the stoichiometry of the coloured products. A $1.0x10^{-3}$ M standard solution of VARD and YOHM and a $1.0x10^{-3}$ solution of *p*-CLA, DDQ and TCNQ were used. A series of solutions was prepared in which the total volume of drug and reagent was constant (2.0 mL). The drugs and reagents were mixed in various proportions and diluted in a 10 mL calibrated flask with acetonitrile solvent. Measure the absorbance at optimum wavelengths after treating each reagent at best time and temperature against a reagent blank following the above mentioned procedure.

Results and Discussion

In the present investigation, we investigated the development of simple, rapid, accurate, reproducible and adequately sensitive spectrophotometric methods for determination VARD and YOHM in bulk powder and pharmaceutical formulation based on the formation of charge-transfer complex of VARD and YOHM as electron-donor with selected π-acceptors (*p*-CLA, DDQ and TCNQ) in acetonitrile. They produce a new band of absorption intensity at a suitable λ_{max} which was characteristic for each complex (Table 1). These new bands were used for a quantitative determination of both drugs. The proposed methods have been successfully applied in pure and in pharmaceutical formulations and favorably comparable with those of the reported methods.

Absorption spectra

The reaction of VARD or YOHM with DDQ results in the formation of an intense orange-red coloured chromogen, which exihibits two maxima at 531 and 461 nm. The 461 nm band, having the highest absorption intensity, was selected for construction of Beer's plot. The predominant colour with DDQ is from the reddish brown radical anion $DDQ^{\cdot-}$, which was probably formed by the dissociation of an original donor-acceptor (DA) complex with VARD and YOHM (Figure 1).

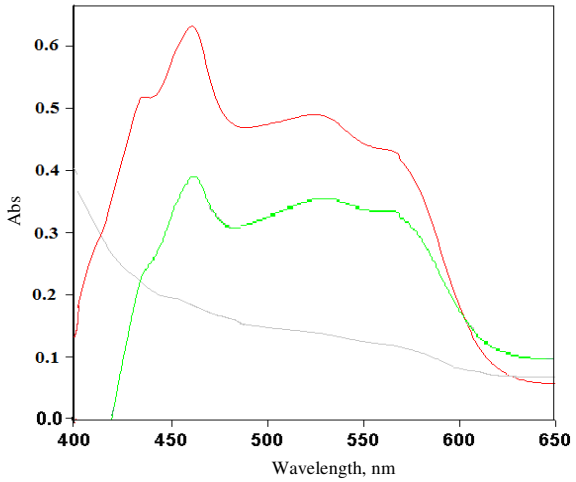


Figure 1. Absorption spectra of reaction products of 100 µg mL^{-1} VARD and 160 µg mL^{-1} YOHM with DDQ ($1.0x10^{-3}$ M) against blank solution

Chloranilic acid (*p*-CA) exists in three ionic forms, the neutral yellow- orange H_2A at very low pH, the dark purple HA^- which is stable at pH 3.0 and a colorless A^{2-}, which is stable at high pH; these transformations are illustrated in the following scheme:

$$H_2A \rightleftharpoons H^+ + HA^- \text{(violet)},$$

$$HA^- \rightleftharpoons H^+ + A^{2-} \text{(colorless)}.$$

Since the interaction of VARD with *p*-CA in acetonitrile forms charge transfer complex gave a violet product (*p*-CLA radical anion) which absorbing maximally at wavelength 535 nm, it might be concluded that HA^- was the form of *p*-CLA involved in the reaction described herein (Figure 2). This compound is considered to be an intermediate molecular association complex which dissociates in the corresponding radical anions in acetonitrile solvent.

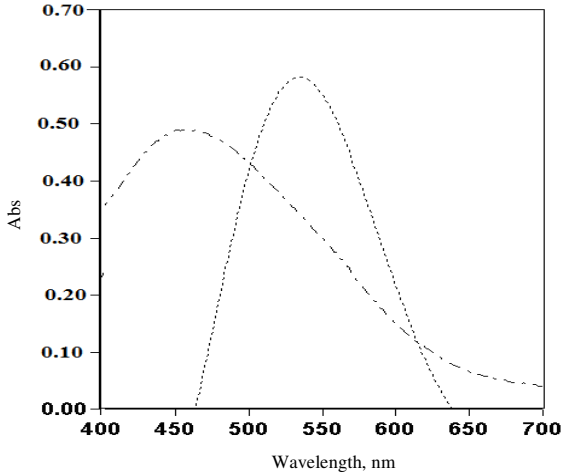


Figure 2. Absorption spectra of reaction products of 200 µg mL^{-1} VARD with *p*-CLA ($1.0x10^{-3}$ M) against blank solution

VARD and YOHM reacts with TCNQ yields intense bluish-green colored radical anion ($TCNQ^{\bullet-}$) in acetonitrile, which exhibits strong absorption maxima at wavelengths 840 and 841 nm, VARD and YOHM, respectively (Figure 3) most probably due to the formation of charge-transfer complexes between the drug acting as *n*-donor (D) or Lewis base and TCNQ, as π-acceptors(A) or Lewis acids[35]:

$$D + A \rightleftharpoons [D \rightarrow A] \xrightarrow{\text{Polar solvent}} D^{\bullet+} + A^{\bullet-}$$

DA complex; radical anion

The dissociation of DA complex is promoted by the high dielectric constant of acetonitrile (ε=37.5). Further support for the assignment was provided by the comparison of the absorption bands with those of the $DDQ^{\bullet-}$, $TCNQ^{\bullet-}$ and *p*-$CLA^{\bullet-}$ radical anions produced by the iodide reduction method. The influence of different parameters on the colour development was studied to determine optimum conditions for the assay procedures.

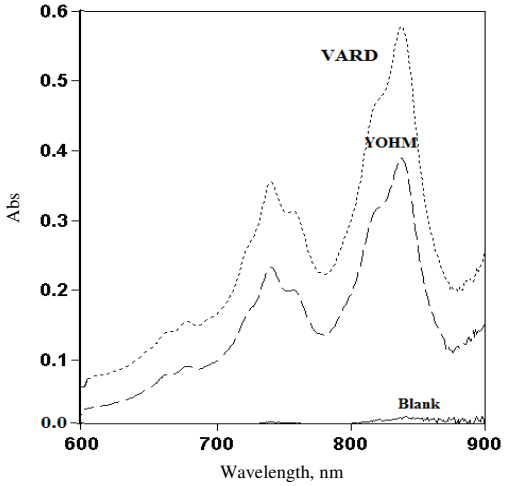


Figure 3. Absorption spectra of reaction products of 40 µgmL^{-1} VARD and 30 µgmL^{-1} YOHM with TCNQ (1.0x10^{-3} M) against blank solution

Optimization of reaction conditions

Effect of solvents

Different solvents such as acetone, methanol, ethanol, dichloromethane, 1,2-dichloroethane, acetonitrile and chloroform were examined. Acetonitrile was found to be the best solvent for all the reagents, because it has a high relative permittivity which ensures the maximum yield of $DDQ^{\bullet-}$, $TCNQ^{\bullet-}$ and *p*-$CLA^{\bullet-}$ species. Of the other solvents examined, chloroform, acetone, dichloromethane and 1,2-dichloroethane are possible substitutes. The formation of $DDQ^{\bullet-}$, $TCNQ^{\bullet-}$ and *p*-$CLA^{\bullet-}$ radicals was possible in methanol or ethanol, however, the colour intensity was lower than in acetonitrile.

Effect of reagent concentration

The optimum concentrations that give maximum colour formation using 2.0 mL of (1.0x10^{-3}M) DDQ in case of VARD or YOHM; 1.5 mL of solution (1.0x10^{-3}M) *p*-CLA solution in acetonitrile was found to be sufficient for the production of maximum and reproducible colour

intensity for VARD. Higher concentrations of the reagent did not affect the colour intensity. When various concentrations of TCNQ were added to affixed concentration of VARD or YOHM, 1.0 mL of ($1.0x10^{-3}$M) solution of TCNQ were found to be sufficient for the production of maximum and reproducible colour intensity (Figure 4). Higher concentrations of reagent did not affect the colour intensity.

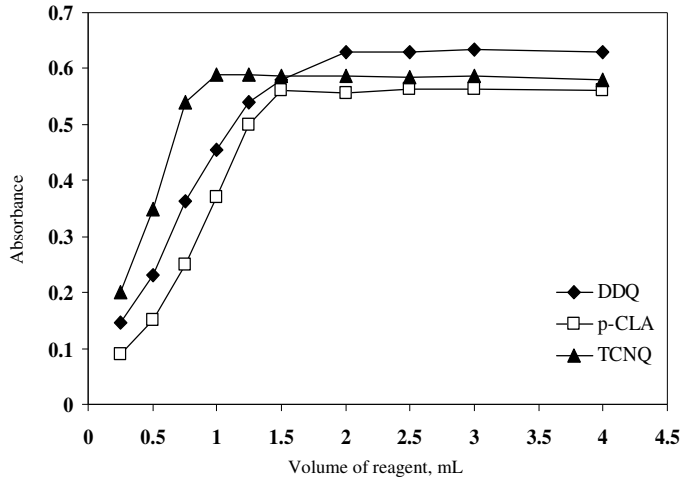


Figure 4. Effect of reagent volume on the formation of VARD complexes with ($1.0x10^{-3}$ M) DDQ, *p*-CLA and TCNQ

Effect of time and temperature

The optimum reaction time was determined by following the colour intensity at ambient temperature (25±2 °C). Complete colour development was attained after 45 min for DDQ and 50 min for *p*-CLA complexes. On raising the temperature to 60±2 °C for 10 and 5.0 min using DDQ with VARD or YOHM and using *p*-CLA with VARD, the complete colour development was obtained. The colour remained stable for 2.0 and 8.0 h for DDQ or *P*-CLA reagent complexes. On using TCNQ, complete colour development was not attained till 90 min; after heating on a water-bath at 60±2 ºC for 15 min for VARD and YOHM, complete colour development was obtained. The colour remained stable for at least 4.0 h for both drugs. The relative sensitivity of the reagents in analytical work can be compared by the apparent molar absorptivity values of the chromogens (Table 1). TCNQ exhibited the most intense band and was therefore selected for all further work. The most important spectral characteristics of the reaction of DDQ, TCNQ and *P*-CLA with the studied drugs are presented in Table 1.

Stoichiometry of the reaction

The stoichiometric ratio of the reactants (drug : reagent) was determined by Job's method[40] of continuous variation for the reaction between VARD or YOHM and (DDQ or *p*-CLA) and TCNQ reagents, which shows that the interaction occurs between an equimolar solution of the drug and the reagents. The result indicated that the charge transfer complex was formed in the ratio of 1:1 (Figure 5). On the basis of the literature data and our experimental results, tentative reaction mechanisms for VARD-TCNQ complex is proposed and given in Scheme 2, respectively.

Method validation

Validation of the described methods for assay of bulk VARD or YOHM was examined via linearity, sensitivity**,** precision, accuracy, repeatability, reproducibility, selectivity and robustness according to ICH guidelines[41] and USP[42].

Table 1. Statistical analysis for determination of VARD and YOHM using (DDQ or *p*-CLA) and TCNQ

Parameters	VARD			YOHM	
	DDQ	*p*-CLA	TCNQ	DDQ	TCNQ
Wavelengths λ_{max}, nm	461	535	840	461	841
Beer's law limits, µg mL^{-1}	5.0-100	10-200	5.0-40	10-160	5.0-30
Molar absorptivity ε, $(L/mol^{-1}\ cm^{-1}) \times 10^3$	3.796	1.765	8.136	5.003	9.312
Sandell's sensitivity, ng cm^{-2}	152.56	328.10	71.18	78.15	41.99
Regression equation [a]					
Slope (b)	0.0064	0.0029	0.0148	0.0023	0.0132
Intercept (a)	0.0015	0.0025	- 0.007	0.0006	-0.004
Correlation coefficient (*r*)	0.9996	0.9997	0.9995	0.9998	0.9995
Mean ± SD	99.83±0.48	100.20±0.64	99.90±0.70	99.80±0.51	100.10±0.43
RSD%	0.48	0.64	0.70	0.51	0.43
RE%	0.50	0.67	0.73	0.53	0.45
LOD, µgmL^{-1} [b]	1.42	1.27	2.46	1.29	2.63
LOQ, µgmL^{-1} [b]	4.73	4.23	8.20	4.30	8.77
Calculated *t*-value [c]	0.88	0.34	0.47	0.79	0.27
Calculated *F*-value [c]	1.88	3.34	4.0	2.54	1.81

[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]LOD, limit of detection; LOQ, limit of quantification; ε, molar absorptivity. [c]The theoretical values of t and F are 2.776 and 5.19, respectively at confidence limit at 95% confidence level and five degrees of freedom (p= 0.05)

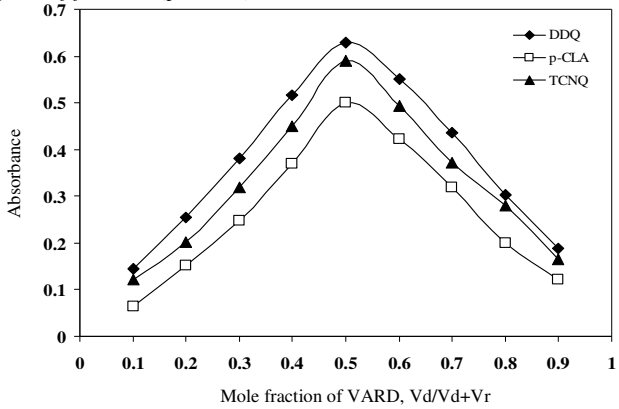
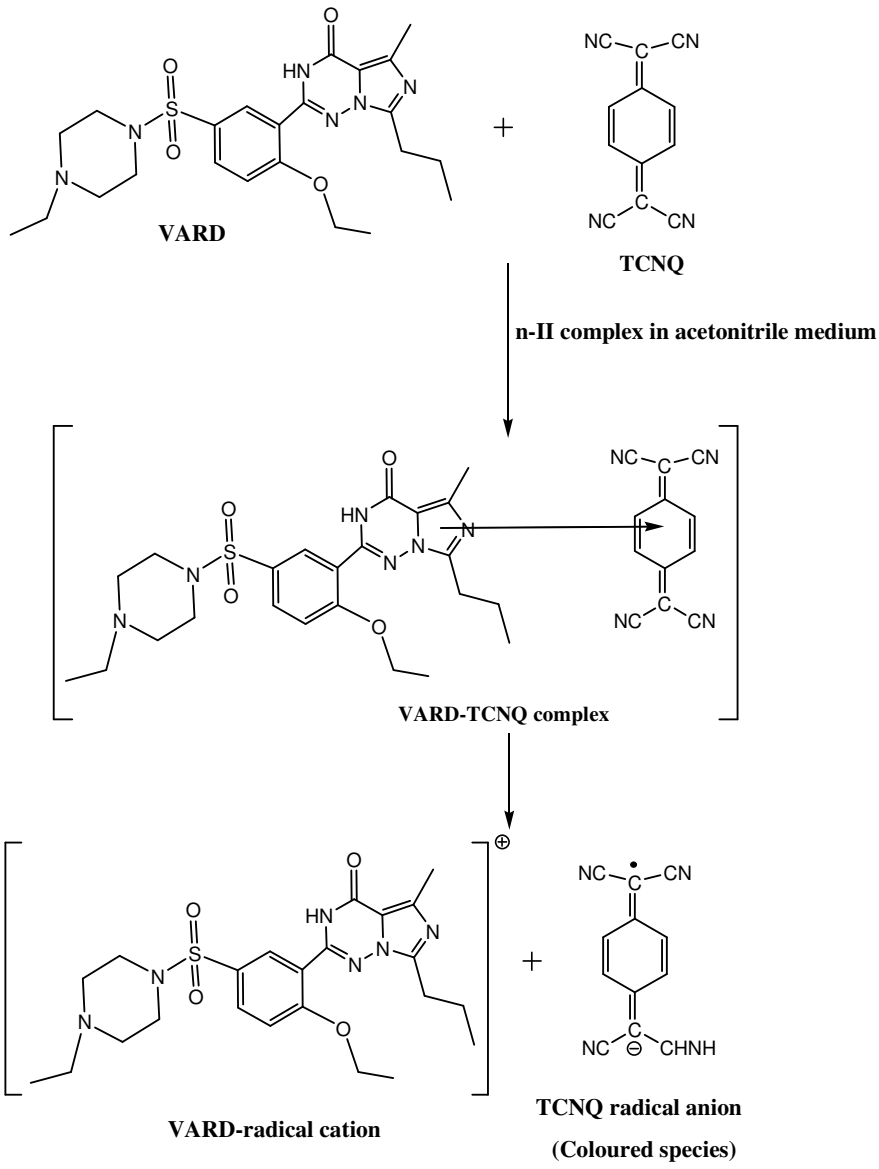


Figure **5**. Job's method of continuous variation graph for the reaction of VARD complexes with DDQ, *p*-CLA and TCNQ at λ=461, 535 and 840 nm, respectively. Total molar concentration = 1.0×10^{-4} M



Scheme 2. Proposed mechanism for the reaction between VARD and TCNQ charge transfer complex

Linearity and sensitivity

Under the specified optimum reaction conditions, the calibration curves for VARD and YOHM with the different analytical reagents employed in the present work were constructed. A linear relation was found to exist between absorbance and the concentration of VARD and YOHM in the ranges given in Table 1. The calibration graph in each case is described by the equation:

$$Y = a + b\,X$$

Where Y= absorbance, a =intercept, b= slope and x=concentration in μg mL^{-1} obtained by the method of least squares[43]. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 1. Sensitivity parameters such as apparent molar absorptivity (ε) and Sandell's sensitivity (Ss) values, the limits of detection and quantification were calculated as per the current ICH guidelines (ICH), are compiled in Table 1 and are indicative of the sensitivity of the methods. The proposed methods were evaluated by statistical analysis between the results achieved from the proposed methods and that of the reported methods[19]. Regarding the calculated Student's *t*-test and variance ratio *F*-test (Table 1), there is no significant difference between the proposed and reported methods regarding accuracy and precision.

The limit of detection (LOD) is defined as the minimum level at which the analyte can be reliably detected for the drug was calculated using the following equation[42,44] and listed in Table 1:

$$\text{LOD} = 3s / k$$

Where *s* is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and *k* is the slope of the calibration graph. In accordance with the formula, the limits of detection were found to be 1.42, 1.27 and 2.46 μg mL^{-1} using DDQ, *p*-CLA and TCNQ, respectively for VARD, whereas for YOHM the limits of detection were found to be 1.29 and 2.63 μg mL^{-1} using DDQ and TCNQ, respectively. The limits of quantification, LOQ, is defined as the lowest concentration that can be measured with acceptable accuracy and precision[42,44],

$$\text{LOQ} = 10\ s / k$$

According to this equation, the limit of quantification was found to be 4.73, 4.23 and 8.20 μg mL^{-1} using DDQ, *p*-CLA and TCNQ, respectively for VARD, whereas for YOHM the limits of detection were found to be 4.30 and 8.77 μg mL^{-1} using DDQ and TCNQ, respectively.

Accuracy and precision

The accuracy and precision of the methods (within-day and between-days) were evaluated by performing six replicate analyses on pure drug solution at three different concentration levels (within the working range). The relative error (RE %), an indicator of accuracy (Table 2) in the range (-0.9-0.6) and within day precision, also called the repeatability, expressed as relative standard deviation (RSD%) was less than 1.38% indicating high accuracy and repeatability of the methods.

$$\%R.E. = \left[\frac{found - taken}{taken} \right] x100$$

The reproducibility of the methods also known as the day-to-day precision was evaluated by performing replicate analyses on pure drug solution at four levels over a period of five days, preparing all solutions afresh. The day-to-day RSD values (Table 2) were less than 1.29% reflecting the usefulness of the methods in routine analysis.

Robustness and ruggedness

The robustness of the methods was evaluated by making small incremental changes in the volume of reagent (±0.2 mL) and time (±1 min), and the effect of the changes were studied on the absorbance of the charge transfer complex. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as %RSD (≤ 1.95%). Method ruggedness was demonstrated having the analysis done by four analysts, and also by a single analyst performing analysis using four different cuvettes. Intermediate precision values (%RSD) in both instances were (≤ 2.0%) indicating acceptable ruggedness.

Table 2. Evaluation of intra-day precision and accuracy for the studied drugs obtained by the proposed methods

Drug	Methods	Added, μgmL^{-1}	Intra-day				Inter-day			
			Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]
VARD	DDQ	20	99.40	0.62	-0.60	19.88±0.129	99.10	0.39	-0.90	19.82±0.081
		50	99.70	0.74	-0.30	49.85±0.387	100.20	0.53	0.20	50.10±0.279
		80	99.90	1.10	-0.10	79.92±0.923	99.80	0.93	-0.30	79.84±0.779
	P-CLA	50	99.20	0.49	-0.80	49.60±0.255	99.40	0.50	-0.60	49.70±0.261
		100	99.60	0.75	-0.40	99.60±0.784	100.30	0.60	0.30	100.30±0.632
		150	100.30	0.98	0.30	150.45±1.548	99.90	1.15	-0.10	149.85±1.809
	TCNQ	10	99.10	0.34	-0.90	9.91±0.035	99.40	0.40	-0.60	9.94±0.042
		20	100.50	0.56	0.50	20.10±0.118	99.60	0.75	-0.40	19.92±0.157
		40	99.50	1.23	-0.50	39.80±0.514	100.30	1.25	0.30	40.12±0.526
YOHM	DDQ	40	99.30	0.57	-0.70	39.72±0.238	99.60	0.42	-0.40	39.84±0.176
		80	100.20	0.80	0.20	80.16±0.673	99.10	0.70	-0.90	79.28±0.582
		120	100.60	1.20	0.60	120.72±1.521	99.20	0.90	-0.80	119.04±0.750
	TCNQ	10	99.20	0.36	-0.80	9.92±0.037	100.10	0.51	0.10	10.01±0.054
		20	99.50	0.70	-0.50	19.90±0.146	99.60	0.82	-0.40	19.92±0.171
		30	99.80	1.38	-0.20	29.94±0.434	99.80	1.29	-0.20	29.94±0.405

[a]Mean of six determination, RSD%, percentage relative standard deviation; R.E%, percentage relative error. [b]Mean ± standard error

Recovery studies by standard addition technique

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. In this study, pre-analyzed tablet powder was spiked with pure VARD or YOHM at different concentration levels and the total was determined by the proposed methods using standard addition technique. The percent recovery of pure VARD and YOHM added was in the range 98.70-100.50% with relative standard deviation of 0.39-1.45 (Table 3) indicating that the recovery was good and revealed that the co-formulated substances did not interfere in the determination. The results of recovery study are compiled in Table 3.

Table 3. Determination of the studied drugs in pharmaceutical dosage forms applying the standard addition technique using the proposed methods

Method	Taken, μgmL^{-1}	Levitra tablets		Yohimbex tablets	
		Added, μgmL^{-1}	Recovery [a] ± RSD %	Added, μgmL^{-1}	Recovery [a] ± RSD %
DDQ	10	-	99.60±0.39	-	99.20±0.42
		10	99.90±0.56	10	99.80±0.65
		20	99.10±0.53	20	100.15±0.50
		40	99.30±0.87	40	98.90±0.62
		60	99.70±1.02	60	99.00±0.67
		80	100.50±1.10	80	99.40±0.94
Mean ± S.D.			99.53±0.55		99.41±0.48
P-CLA	10	-	99.80±0.56		
		10	99.10±0.75		
		20	99.00±0.57		
		40	98.70±0.76		
		50	100.50±0.90		
		60	99.30±1.45		
Mean± S.D.			99.40±0.65		
TCNQ	5.0	-	99.00±0.42	-	99.40±0.58
		5.0	98.80±0.49	5.0	99.70±0.75
		10	100.40±0.61	10	99.90±0.60
		15	99.20±1.13	15	100.50±0.85
		20	99.10±1.40	20	100.10±1.27
		30	99.60±1.28	25	99.20±0.96
Mean ± S.D.			99.35±0.58		99.80±0.47

[a]*Average of six determinations*

Interference studies

The studied drugs (VARD and YOHM) were determined in the presence of possible excipients and additives such as lactose, microcrystalline cellulose, sodium starch glycolate and magnesium stearate. Under the experimental conditions employed, to a known amount of drug, excipients in different concentrations were added and studied. Excipients do not interfere with the assay. In addition, recoveries in most cases were around 100% and the lower values of the RSD (≤ 2.0%) indicate the good precision of the methods.

Regarding the interference of the excipients and additives usually presented in pharmaceutical formulation and interference due to the degradation products of VARD and YOHM, the energy of the charge transfer (E_{CT}) depends on the ionization potential (I_P) of the

donor and the electron affinity of the acceptor (E_A), hence the λmax values of the other π-donors mostly differ from that of the investigated compounds if they are able to form CT complexes. Preliminary experiments showed that all additives, excipients and degradate products did not form CT complexes with the studied acceptors indicating the high selectivity of the proposed methods and applicability to use for routine determination in pure and in dosage forms.

Application of the proposed methods to the analysis of tablets

In order to evaluate the analytical applicability of the proposed methods to the quantification of VARD and YOHM in commercial tablets, the results obtained by the proposed methods were compared with those of the reference methods for VARD[19] and YOHM[34] by applying Student's *t*-test for accuracy and *F*-test for precision. The results (Table 4) show that the Student's *t*-and *F*-values at 95% confidence level are less than the theoretical values, indicating that there is a good agreement between the results obtained by the proposed methods and the reference method with respect to accuracy and precision.

Table 4. Determination of the studied drugs in pharmaceutical dosage forms

Sample	Reported methods	Recovery [a] ± RSD %		
		DDQ	*P*-CLA	TCNQ
Levitra tablets (10 mg VARD/tab)	100.02±0.55	99.70±0.62	99.85±0.57	100.15±0.67
t [b]		0.81	0.45	0.76
F [b]		1.27	1.07	1.48
Yohimbex tablets (5.4 mg YOHM/tab)	99.26 ± 0.65	99.40±0.80		99.56±0.74
t [b]		0.28		0.64
F [b]		1.51		1.30

[a]Average of six determinations. [b]The theoretical values of t and F are 2.776 and 5.19, respectively at confidence limit at 95% confidence level and five degrees of freedom (p= 0.05)

Conclusion

The present study described the successful evaluation of some π acceptors (DDQ or *p*-CLA) and TCNQ as analytical reagents in the development of simple and rapid charge transfer complexation spectrophotometric methods for the accurate determination of VARD and YOHM in drug substance and pharmaceutical formulations. The methods described herein have many advantages: they do not need expensive sophisticated apparatus, are simple and rapid with high sensitivity. The proposed methods used inexpensive reagents with excellent shelf life and they complied with the validation scheme of the ICH and can therefore be used for quality control and routine analysis.

Conflicts of interests

All authors declare that they have no conflicts of interests

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