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Development of New 96-Microwell-Based Spectrophotometric Assay with High-Throughput and its Application in Pharmaceutical Quality Control of Varenicline

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Abstract: This study describes, for the first, the development of a new 96-micorwell-based spectrophotometric assay with high-throughput for the pharmaceutical quality control of varenicline (VRC). The reaction between VRC and 1,2-naphthoquinone-4-sulphonate (NQS) as a chromogenic reagent was investigated. In alkaline medium, a red-colored product exhibiting maximum absorption peak at 490 nm was produced. This color-developing reaction was employed, for the first time, in the development of the proposed assay. The reaction was carried out in 96-microwell plate and the absorbance of the colored-product was measured by microwell plate absorbance reader at 490 nm. The optimized reaction conditions were established; under which, Beer's law was obeyed in the range of 10 - 100 μg ml^{-1} of VRC. The limits of detection and quantification were 3.1 and 10.2 μg ml^{-1}, respectively. The assay showed high precision as the values of relative standard deviations (RSD) did not exceed 2%. The proposed assay was applied successfully for the determination of VRC in its pharmaceutical tablets with good accuracy and precisions; the label claim percentages were 100.80 ± 1.62%. The proposed assay is practical and valuable in terms of its routine application in pharmaceutical quality control laboratories.

Key words: Varenicline · 1,2-Naphthoquinone-4-sulphonate · 96-Microwell-based assay · Photometry · High-throughput · Pharmaceutical analysis.

INTRODUCTION

Varenicline (VRC, Fig. 1); 7,8,9,10-tetrahydro-6,10-methano-6*H*-azepino[4,5-*g*]quinoxaline (2*R*,3*R*), is a novel agent that is a centrally acting as a highly selective partial agonist for the nicotinic acetylcholine receptor [1]. VRC has mixed agonistic-antagonistic properties, thus it has the therapeutic benefit of relieving the symptoms of nicotine withdrawal and cigarette craving during abstinence while blocking the reinforcing effect of nicotine in those who lapse [2-4]. VRC tartrate has been approved by the USA-FDA as an aid to smoking cessation [5]. The approved regime of VRC is 1 mg for 12 weeks, starting with a one-week titration period [6].

The effective and safe therapy with VRC is basically depending on the quality of its pharmaceutical preparations (tablets) and assessing its concentrations in tablets for the purposes of quality control. As well, the therapeutic benefits profile of VRC is anticipated to encourage the development of new pharmaceutical

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preparations for VRC. As a consequence, there is an increasing demand for a proper analytical method for quality assurance of VRC bulk drug and its finished pharmaceutical formulations. VRC has not yet been officially described in any pharmacopoeia. In literature, only two chromatographic methods have been found describing the quality control (QC) of VRC [7,8].

Spectrophotometry has considerable importance in drug analysis and photometric methods are extensively applied in pharmaceutical QC for the determination of active substances in bulk drugs and pharmaceutical preparations [9]. The importance of spectrophotometric methods in the field of pharmaceutical QC has greatly increased, due to the fact that these methods can be very readily automated. Automated analyzers equipped with photometric detection are widey used for the serial analysis of pharmaceutical preparations, especially for studying the contents uniformity and dissolution characteristics of the solid dosage forms [9]. Unfortunately, no spectrophotometric method was reported for determination of VRC. The present study described the development of a new 96-microwell-based spectrophotometric assay with high-throughput for QC of VRC. The method was based on the reaction of VRC with 1,2-naphthoquinone-4-sulphonate (NQS) in alkaline buffered medium to produce a red-colored product. The reaction was carried out in 96-microwell assay plate and the absorbance was measured by microwell absorbance plate reader.

Experimental

Apparatus: Microwell-plate absorbance reader (ELx 808, Bio-Tek Instruments Inc. Winooski, USA) was used for all the measurements in 96-microwell plates. Double beam V-530 (JASCO Ltd. Kyoto, Japan) ultraviolet-visible spectrophotometer with matched 1-cm quartz cells was used for all the spectrophotometric measurements. pH meter, model 350 (Bibby Scientific Ltd. T/As Jenway, Essex, England). 96-Microwell plates were a product of Corning/Costar Inc. (Cambridge, USA). Finnpipette adjustable 8-channel-pipette was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Reagents and Materials: Varenicline tartarate standard with claimed purity of 99.6% was purchased from Weihua Pharma Co. Ltd. (Zhejiang, China). Aqueous solution (0.5%, w/v) of NQS (Aldrich Chemical Co. St. Louis, USA) was freshly prepared and protected from light during use. Buffer solutions (Robinson's, Clark's, tris, borate and phosphate) were prepared as previously described [10]. Champix® tablets (Pfizer Inc, New York, USA), labeled to contain 1 mg VRC per tablet was obtained from the local market. Double distilled water was obtained through WSC-85 water purification system (Hamilton Laboratory Glass Ltd. Kent, USA) and used throughout the work. All solvents and materials used throughout this study were of analytical grade.

Preparation of VRC Standard Solution: An accurately weighed amount (20 mg) of VRC was quantitatively transferred into a 10-ml calibrated flask, dissolved in 5 ml water, completed to volume with the same solvent to obtain a stock solution of 2 mg ml^{-1}. The stock solution was found to be stable for at least two weeks when kept in a refrigerator. The stock solution was further diluted with water to obtain working solutions in the range of 10 - 100 µg ml^{-1}.

Preparation of Tablets Sample Solution: Ten Champix® tablets were weighed and crushed to a fine powder. An accurately weighed quantity of the tablet powder equivalent to 20 mg of VRC was transferred into a 10-ml calibrated flask and dissolved in about 5 ml of water. The contents of the flask were swirled, sonicated for 20 min and then completed to volume with water. The contents were mixed well and centrifuged at 6000 rpm for 10 min; the supernatant was separated and filtered, the first portion of the filtrate was rejected. The filtered solution was diluted quantitatively with water to obtain working solutions in the range of 10 - 100 µg ml^{-1}.

General Recommended Procedures: An accurately measured volume (50 µl) of VRC solution containing 10 - 100 µg ml^{-1} was transferred into wells of 96-microwell plate. A 50 µl of tris buffer solution of pH 8.5 and 100 µl of NQS solution (0.5%, w/v) were added. The reaction solution was allowed to proceed for 5 min at room temperature (25±2°C) and the resulting color was measured at 490 nm.

Determination of Stoichiometric Ratio by Job's Method: The Job's method [11] of continuous variation was employed. Master equimolar (1×10^{-4} M) solutions of VRC and NQS were prepared. Series of 200 µl portions of the master solutions of VRC and NQS were made up comprising different complementary proportions (0:200, 10:190,..., 190:10, 200:0, inclusive) in microwells containing 50 µl of tris buffer solution (pH 8.5). The solutions were further manipulated as described under the general recommended procedures.

Procedures for Limiting Logarithmic Method: The limiting logarithmic method [12] was employed. Two sets of experiments were carried out employing the general recommended procedures described above. The first set of experiments was carried using varying concentrations of NQS (2×10^{-3} - 1×10^{-2} M) at a fixed VRC concentration (0.2×10^{-3} M). The second set of experiments was carried using varying concentrations of VRC (0.5×10^{-4} - 0.5×10^{-3} M) at a fixed concentration of NQS (2×10^{-2} M). The logarithms of the obtained absorbances for the reaction of VRC with NQS were plotted as a function of the logarithms of the concentrations of the NQS reagent and VRC in the first and second sets of experiments. The slopes of the fitting lines in both sets of experiments were calculated.

RESULTS AND DISCUSSION

Strategy for Assay Development and its Design: The chemical structure of VRC and its absorption spectrum are given in Fig. 1A. It is obvious from the spectrum that VRC exhibits a maximum absorption peak (λ_{max}) at 237. Because of the blue shifted λ_{max} of VRC, its determination in the pharmaceutical formulations (tablets) based on the direct measurement of its absorption for ultraviolet light is susceptible to potential interferences from the co-extracted tablet excipients. Therefore, derivatization of VRC to a more red-shifted light-absoping derivative was necessary. VRC contains a secondary amino group for which many chromogenic reagents could be used for color-producing reactions. These reactions include formation of colored charge-transfer complex with electron acceptor [13], formation of ion-pair associates with pairing reagents [14-16] and formation of condensation product with a chromogenic reagent [17]. However, these methods are usually associated with some major drawbacks such as laborious multiple extraction steps in the analysis by ion-pair based methods [14-16], or in preparation of the free base of the drug prior to the analysis by charge-transfer-based methods [13] and long reaction time, thus the procedure is time-consuming [17]. In previous studies, Darwish *et al.* [18-21] has demonstrated that NQS is a valuable color-developing reagent in the development of simple spectrophotometric methods for the determination of many pharmaceutical amines in the form of their acid salts. For these reasons, the present study was devoted to investigate the reaction between NQS and VRC and employed this color reaction in the development of a new simple and rapid spectrophotometric method with high-throughput for determination of VRC in its tablets.

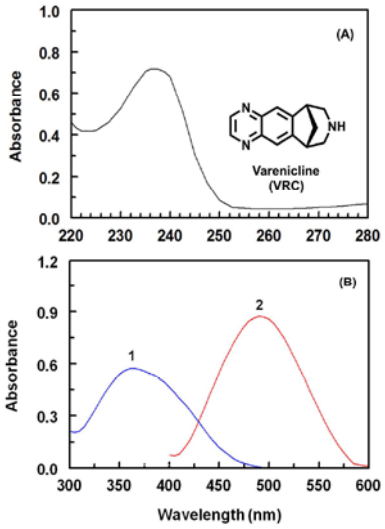

Fig. 1: Panel A: Chemical structure of varenicline (VRC) and absorption spectrum of its aqueous solution (10 µg ml^{-1}) against water. Panel B: The absorption spectrum of NQS solution (0.5%, w/v; line 1) and its reaction product with VRC (50 µg ml^{-1}; line 2).

The reaction between VRC and NQS was performed and the absorption spectrum of the reaction product was recorded against reagent blank. The product was orange red-colored exhibiting λ_{max} at 490 nm (Fig. 1B). Obviously, the λ_{max} of VRC-NQS derivative was significantly red-shifted from the underivatized VRC by 253 nm. This shift enables the measurements in the visible region and eliminates the potential interference from tablet excipients.

The proposed assay was designed to employ 96-microwell assay plate as the reaction between VRC and NQS was carried out in microwells of the assay plate (200-µl total reaction volume). The solutions were dispensed by 8-channel pipette and the absorbance of the colored-reaction product was measured by 96-microwell-plate absorbance reader. The 96-microwell design of the proposed assay was considered based on the previous success of Darwish *et al.* [22,23] in the employment of this methodology for the high-throughput analysis of some other pharmaceuticals. The involved studies will be described in the following paragraphs.

Optimization of Reaction Conditions: The optimization of experimental conditions affecting the reaction in the 96-microwell format was investigated by altering each reaction variable in a turn while keeping the others constant. All the measurements were carried out by the plate reader at 490 nm.

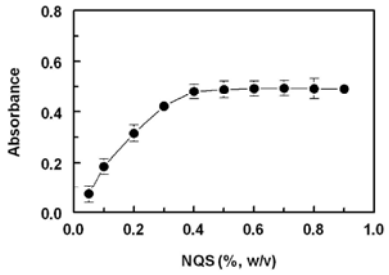

Fig. 2: Effect of NQS concentration on its reaction with VRC (50 µg ml^{-1}) .

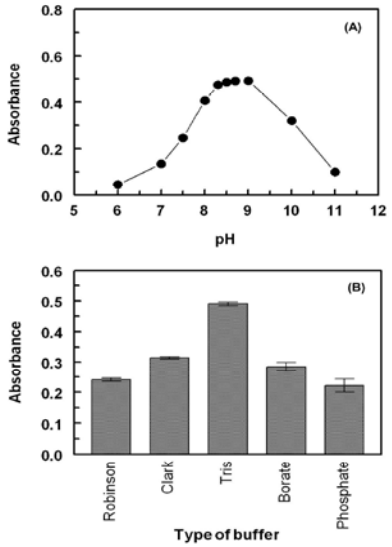

Fig. 3: Effect of pH (A) and type of buffer solution of pH 8.5 (B) on the reaction of VRC (50 µg ml^{-1}) with NQS (0.5%, w/v)

Effect of NQS Concentration: Studying the effect of NQS concentration on its reaction with VRC revealed that the reaction was dependent on the NQS concentration as the readings increased with the increase in the reagent concentration (Fig. 2). The highest readings were attained at a concentration range of 0.4-0.9% (w/v). A concentration of 0.5% (w/v) was used in all the subsequent experiments.

Effect of Ph and Buffer Components: The influence of pH on the reaction of VRC with NQS was investigated by carrying out the reaction in buffer solution of varying pH values. The results revealed that VRC has a quite low reactivity with NQS in acidic pH (Fig. 3A). This was possibly due to the formation of acid salt with the secondary amino group of VRC, thus it decreases its nucleophilic substitution affinity. As the pH increased, the readings increased and their maximum values were attained in pH range of 8.2-9. This high reactivity was attributed to the fact that alkaline pH keep the amino group of VRC as it is, thus facilitating the nucleophilic substitution with NQS reagent. At higher pH, a decrease in the readings occurred. This was attributed probably to the increase in the amount of hydroxide ion that holds back the reaction of VRC with NQS and the instability of NQS reagent. All subsequent experiments were carried out at pH 8.5.

In order to investigate the effect of buffer components on the reaction, different buffer solutions adjusted at pH 9 were tested: Robinson's, Clark's, tris, borate and phosphate buffers. The highest readings were obtained when tris buffer was used (Fig. 3B), thus it was used in all the subsequent experiments.

Effect of Temperature and Time: The effect of temperature on the reaction was studied by carrying out the reaction at room temperature (25 ± 2 °C) and at varying elevated temperatures (25-60 °C). The results revealed that the reaction of VRC with NQS has not been affected by temperature in the range of 25-40°C; however, at higher temperature the reaction was negatively affected (Fig. 4). Therefore, the subsequent experiments were carried out at room temperature (25 ± 2 °C).

In order to determine the optimum time that is required for completion the reaction, it was allowed to proceed at room temperature for varying periods of time. It was found that the reaction was almost instantaneous as it goes to almost completion within 2 min (Fig. 4); however, for higher precision readings, the reaction was allowed to proceed for 5 min in all the subsequent experiments.

Effect of Solvent: In order to select the most appropriate solvent for carrying out the reaction of NQS with VRC, different solvents (water, methanol, ethanol, isopropanaol, acetonitrile and dioxane) were tested. Upon using water, transparent solution was obtained indicating the solubility of the VRC-NQS product in water and the possibility of using water as a solvent. Upon comparing the solvents, the highest readings were obtained when ethanol was used for dilution (Fig. 5). However, the use of organic solvents leads to high analysis cost and more importantly, the incidence of exposure of the analysts to the toxic effects of the organic solvents [24-28]. Therefore, water was used as a solvent in the subsequent investigation on the expense of the higher sensitivity, which was not a main concern in the present study as the assay described herein was devoted to the analysis of VRC in its bulk and/or dosage forms that does not require highly sensitive assay.

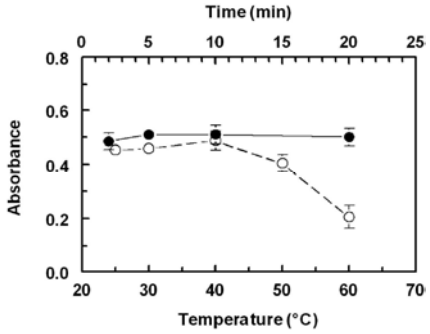

Fig. 4: Effect of temperature (○) and time (●) on the reaction of VRC (50 mg ml^{-1}) with NQS (0.5%, w/v).

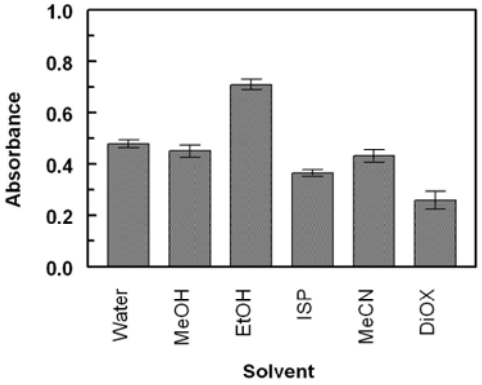

Fig. 5: Effect of diluting solvent on the absorbances of the reaction product between VRC (50 µg ml^{-1}) with NQS (0.5%, w/v). Abbreviated solvents are: water, methanol (MeOH), ethanol (EtOH), isopropanol (ISP), acetonitrile (MeCN), and dioxane (DiOX).

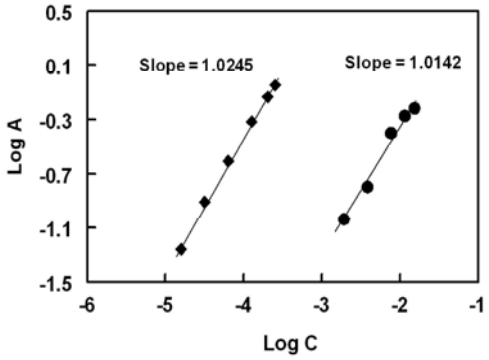

Fig. 6: Limiting logarithmic plot for molar reactivity of VRC with NQS. C and A are the concentration and absorbance, respectively. For generating the first line (♦), [NQS]: 2×10^{-2}; [VRC]: 0.5×10^{-4} - 0.5×10^{-3} M. For generating the second line (•), [NQS]: 2×10^{-3} - 1×10^{-2} M; [VRC]: 2×10^{-3}M.

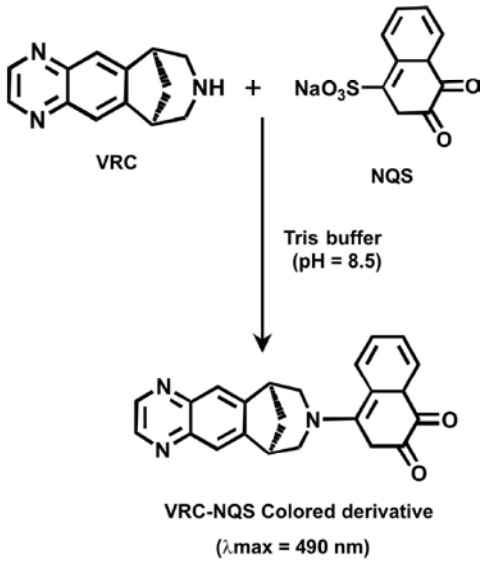

Fig. 7: Scheme for the reaction pathway of VRC with NQS.

Stability of the Chromogen: The effect of time on the stability of the VRC-NQS chromogen was studied by following the absorption intensity of the reaction solution at different time intervals. It was found that the absorbance of the chromogen remains stable for at least 1 h (data not shown). This allowed comfortable analytical processing of large batches of samples. This gives a high throughput property to the proposed method when applied for analysis of large number of samples in quality control.

A summary for the optimum conditions for the reaction between VRC and NQS is given in Table 1.

Stoichiometry, Kinetics and Mechanism of the Reaction: Under the optimum conditions (Table 1), the stoichiometry of the reaction between VRC and NQS was investigated by Job's method. The symmetrical bell shape of Job's plot indicated that the VRC:NQS ratio was 1:1. In the limiting logarithmic method, two straight lines were obtained (Fig. 6). The slopes of these lines were comparable (1.0245 and 1.0142), confirming the 1:1 ratio for the reaction. Based on this ratio, the reaction pathway was postulated to be proceeded as shown in Fig. 7.

Under the optimum conditions, the signal-time curves for the reactions at varying concentrations of VRC (0.5×10^{-4} - 1.5×10^{-4} M) for the reaction with a fixed concentration of NQS (1.92×10^{-3} M) were generated. The initial reaction rates (K) were determined from the slopes of these curves. The logarithms of the reaction rates (Log K) were plotted as a function of logarithms of

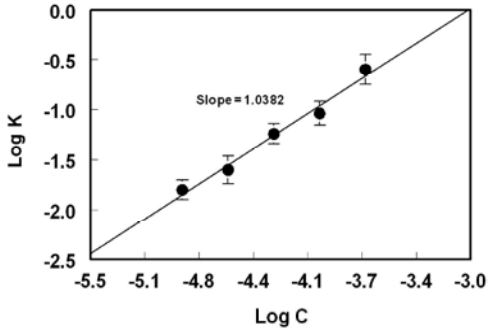

Fig. 8: Linear plot for log C versus log K for the kinetic reaction of VRC with NQS. C is the VRC concentration (0.5×10^{-4} - 1.5×10^{-4} M) and K is the reaction rate (second^{-1}).

VRC concentrations (log *C*). A straight line with slope value ~ 1 was obtained (Fig. 8) upon fitting the data to the following equation:

$$\text{Log } K = \log K' + n \log C$$

where *K* is reaction rate, *K*' is the rate constant, C is the molar concentration of VRC and n(slope) of regression line) is the order of the reaction. The value of the slope (~1) confirmed that the reaction was first order (Fig. 8). However under the optimized reaction conditions, the concentrations of NQS were in much more excess than that of VRC in the reaction solution. Therefore, the reaction was regarded as pseudo-first order reaction.

Validation of the Proposed Assay: Assay validation was conducted according to The International Conference of Harmonization (ICH) guidelines for validation of analytical procedures [29].

Calibration and Sensitivity: Under the optimum reaction conditions (Table 1), the calibration curve for the determination of VRC by its reaction with NQS was constructed by plotting the absorbances as a function of the corresponding concentrations. The regression equation for the results was A = 0.002 + 0.097 C (r = 0.9980), where A is the absorbance at 490 nm, C is the concentration of VRC in µg ml^{-1} in the range of 10 - 100 µg ml^{-1} and r is the correlation coefficient. The limit of detection (LOD) and limit of quantification (LOQ) were determined using the following formula was used: LOD or LOQ= ×SDa/b, where ×= 3.3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept and b is the slope. The LOD and LOQ were found to be 3.1 and 10.2 µg ml^{-1}, respectively.

Table 1: Summary for the optimization of variables affecting the reaction of VRC with NQS reagent employed in the development of the proposed 96-microwell-based spectrophotometric assay for VRC.

Variable	Studied range	Optimum
NQS concentration (%, w/v)	0.05 - 0.9	0.5
pH	6 - 11	8.5
Buffer solution	Differenta	Tris
Temperature (°C)	25 - 60	25
Time (min)	2 - 20	5
Solvent	Different[b]	Water
Chromogen stability time (min)	5 - 90	60
Measuring wavelength (nm)	400 - 600	490

[a]Buffer solutions were: Robinson's, Clark's, tris, borate and phosphate.
[b]Solvents were: water, methanol, ethanol, isopropanaol, acetonitrile and dioxane.

Table 2: Quantitative parameters for the analytical performance of the proposed 96-microwell-based spectrophotometric assay for VRC.

Parameter	Value
Linear range (µg ml^{-1})	3 - 100
Intercept (a)	0.002
Slope	0.097
Correlation coefficient	0.9980
Limit of detection, LOD (µg ml^{-1})	3.1
Limit of quantification, LOQ (µg ml^{-1})	10.2

Table 3: Precision of the proposed 96-microwell-based spectrophotometric assay at different concentrations of VRC.

VRC Concentration (µg ml^{-1})	Relative standard deviation	
	Intra-assay, n = 6	Inter-assays, n = 6
20	1.25	1.87
40	0.48	0.93
80	1.54	1.92

The parameters for the analytical performance of the proposed method are summarized in (Table 2).

Precision and Accuracy: The intra-assay precision of the proposed method was determined on samples of drug solutions at varying concentration levels (Table 3) by analyzing 6 replicates of each concentration as a batch in a single assay run. The inter-assay precision was determined by analysis the same samples as duplicates in three consecutive days. The relative standard deviations (RSD) did not exceed 2 % (Table 3) proving the high precision of the proposed assay for the routine application in the analysis of VRC in quality control laboratories.

Accuracy and Interference Liabilities: The accuracy of the proposed method was evaluated by the recovery studies. The recovery values were 98.54 - 100.42 ± 1.05 - 1.49% (Table 4), indicating the accuracy of the proposed assay. Before proceeding with the analysis of VRC in its dosage forms, interference liabilities were carried out to

Table 4: Recovery studies for determination of VRC by the proposed 96-microwell-based spectrophotometric assay.

	VRC		
Sample number	Nominal (μg ml^{-1})	Found (μg ml^{-1})[a]	Recovery (% ± SD)[a]
1	20	19.71	98.54 ± 1.26
2	40	40.17	100.42 ± 1.05
3	80	79.66	99.58 ± 1.49

[a]Values are mean of three determinations.

Table 5: Analysis of VRC in presence of the excipients that are present in its tablets by the proposed 96-microwell-based spectrophotometric assay.

Excipient	Recovery (% ± SD)[a]
Microcrystalline cellulose (10)[a]	101.05 ± 1.28
Crosscarmellose sodium (10)	97.69 ± 1.91
Calcium hydrogen phosphate anhydrous (5)	98.74 ± 1.26
Silica-colloidal anhydrous (5)	99.21 ± 0.84
Magnesium stearate (5)	101.24 ± 1.05
Average ± SD	99.47 ± 1.19

[a]Values are mean of three determinations.

[b]Figures in parenthesis are the amounts (in mg) that were added per 1 mg of VRC.

Table 6: Influence of small variations in the assay conditions on the analytical performance of the proposed 96-microwell-based spectrophotometric assay for VRC.

Parameters	Recovery (% ± SD)[a]
NQS concentration (%, w/v)	
0.4	99.24 ± 1.22
0.6	101.05 ± 1.05
Buffer solution (pH)	
8.3	97.82 ± 1.27
8.7	100.53 ± 1.91
Reaction time (min)	
3	97.18 ± 1.08
8	101.54 ± 1.52

[a]Values are mean of 3 determinations.

explore the effect of inactive ingredients that might be added during VRC formulation [30]. Samples were prepared by mixing known amount (1 mg) of VRC with 10 mg of microcrystalline cellulose, 10 mg of calcium hydrogen phosphate anhydrous, 5 mg of croscarmellose sodium, 5 mg of silica-colloidal anhydrous and 5 mg magnesium stearate. These laboratory-prepared samples were analyzed by the proposed method applying the general recommended procedure. The average recovery value was of 99.47 ± 1.19% (Table 5). These data confirmed the absence of interference from any of the inactive ingredients with the determination of VRC by the proposed method.

Robustness: Robustness was examined by evaluating the influence of small variation in the assay variables on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged and the recovery percentage was calculated each time. It was found that small variation in one of the parameters did not significantly affect the procedures; recovery values were 97.18 - 100.54% ± 1.05 - 1.91% (Table 6). This indicated the reliability of the proposed assay during its routine application for the analysis of VRC.

Application for Analysis of VRC in Tablets: It is evident from the above-mentioned results that the proposed method gave satisfactory results with VRC in bulk powder. Thus, its pharmaceutical dosage form (Champix® tablets) was subjected to the analysis of their VRC contents by the proposed method. The percentage found from the label claim was 100.8 ± 1.62%. This result was compared with those obtained by a reported method [8]; the label claim percentage was 100.1 ± 1.3%, with respect to the accuracy (by t-test) and precision (by F-test). It was found that the calculated t-and F-values (1.71 and 1.91 for t- and F-value, respectively) were lower than the tabulated ones (2.31 and 6.61 for t- and F-value, respectively). This indicated that there were no significant differences between the means and variance between the two methods in terms of the accuracy and precision.

CONCLUSIONS

This study described the successful development of a novel 96-microwell-based spectrophotometric assay for the accurate determination of VRC in its bulk and tablets based on its derivatization with NQS as a chromogenic analytical reagent. The assay described herein offered the following advantages:

- Use of an inexpensive, stable reagent with excellent shelf life and available in any pharmaceutical QC laboratory.
- Use of minimum volumes of reagent and organic solvents (environmentally friendly "Green" approach), accordingly reduction in the analysis cost and exposures of the analysts to the toxic effects of organic solvents.
- Providing a high throughput analytical methodology that can facilitate the processing of large number of samples in a relatively short time. This property was attributed to the use of multi-channel pipettes for

efficient dispensing of the solutions, carrying out the analytical reaction in 96-well plates (as reaction vessels) and measuring the color signals in the 96 wells at ~ 30 seconds by the plate reader.

- Although the proposed assay was developed and validated for VRC, however, it is also anticipated that the same methodology could be used for essentially any analyte that can exhibit the same substitution reaction with NQS reagent.

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REFERENCES

1. Rollema, H., L.K. Chambers, J.W. Coe, J. Glowa, R.S. Hurst, L.A. Lebel, Y. Lu, R.S. Mansbach, R.J. Mather, C.C. Rovetti, S.B. Sands, E. Schaeffer, D.W. Schulz, F.D. Tingley III, K.E. Williams, 2007. Pharmacological profile of the alpha4beta2 nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. Neuropharmacology, 52(3): 985-994.
2. Dani, J.A. and M. De Biasi, 2001. Cellular mechanisms of nicotine addiction. Pharmacology Biochemistry and Behavior, 70(4): 439-446.
3. Picciotto, M.R., M. Zoli, R. Rimondini, C. Léna, L.M. Marubio, E.M. Pich, K. Fuxe, J.P. Changeux, 1998. Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. Nature, 391(1): 173^{-1}77.
4. Tapper, A.R., S.L. McKinney, R. Nashmi, J. Schwarz, P. Deshpande, C. Labarca, P. Whiteaker, M.J. Marks, A.C. Collins, H.A. Lester, 2004. Nicotine activation of alpha4* receptors: sufficient for reward, tolerance and sensitization. Science, 306 (5698): 1029-1032.
5. Zieler-Brown, L., J. Kyle, 2007. Oral varenicline for smoking cessation. The Annals of Pharmacotherapy, 41(1): 95- 99.
6. Chantix, package Insert. New York, NY: Pfizer Inc; 2008.
7. Satheesh, B., S. Kumarpulluru, V. Raghavan, D. Saravanan, 2010. UPLC separation and quantification of related substances of varenicline tartarate tablet. Acta Chromatographica, 22(2): 207-218.
8. Kadi, A., M.S. Mohamed, M.G. Kassem, I.A. Darwish, 2011. A validated stability-indicating HPLC method for determination of varenicline in bulk and tablets. Chemistry Central Journal, 5(30): 1-6.
9. Görög, S., 1994. Ultraviolet-Visible Spectrophotometry in Pharmaceutical Analysis, CRC Press, New York.
10. Pesez, M. and J. Bartos, 1974. Colorimetric and Fluorimetric Analysis of Organic Compounds and Drugs. Marcel Dekker Inc. New York, pp: 628-630.
11. Job, P., 1964. Advanced Physicochemical Experiments, 2nd ed. Oliner and Boyd, Edinburgh, pp: 54.
12. Rose, J., 1964. Advanced Physicochemical Experiments, Pitman, London.
13. Foster, R., 1969. Organic charge-transfer complexes. London, New York, Academic Press, pp: 470.
14. Starczewska, B., A. Jasiñska and B. Białous, 2003. Study and analytical application of ion-pair formation in the system fluoxetine-pyrocatechol violet and fluvoxamine-pyrocatechol violet. Die Pharmazie, 58(4): 245-248.
15. Starczewska, B. and K. Mielech, 2000. Application of chrome azurol S for the extractive spectrophotometric determination of fluoxetine and fluvoxamine. Journal of Pharmaceutical and Biomedical Analysis, 23(2-3): 243-247.
16. Starczewska, B., H. Puzanowska-Tarasiewicz and K. Baranowska, 2000. Investigation and analytical application of the reactions of eriochrome cyanine R with fluvoxamine and fluoxetine. Journal of Pharmaceutical & Biomedical Anal., 23(2-3): 477-481.
17. Onal, A., S.E. Kepekçi and A.A. Oztunç, 2005. Spectrophotometric methods for the determination of the antidepressant drug paroxetine hydrochloride in tablets. Journal AOAC International, 88(1): 490-495.
18 Darwish, I.A., 2005. Kinetic spectrophotometric methods for determination of trimetazidine dihydrochloride. Analytica Chimica Acta, 551(1-2): 222-231.
19. Darwish, I.A., H.H. Abdine, S.M. Amer and L.I. Al-Rayes, 2009. Simple spectrophotometric method for the determination of paroxetine in tablets using 1,2-naphthoquinone-4-sulphonate as a chromogenic reagent. International Journal of Analytical Chemistry,(237601): 1-8.
20. Darwish, I.A., H.H. Abdine, S.M. Amer and L.I. Al-Rayes, 2009. New spectrophotometric and fluorimetric methods for determination of fluoxetine in pharmaceutical formulations. International Journal of

Analytical Chemistry, (257309): 1-9.

21. Darwish, I.A., H.H. Abdine, S.M. Amer and L.I. Al-Rayes, 2009. Spectrophotometric Study for the Reaction of Fluvoxamine 1,2-naphthoquinone-4-sulphonate: Kinetic, Mechanism and Use for Determination of Fluvoxamine in its Dosage Forms. Spectrochimica Acta A, 72(4): 897-902.
22. Darwish, I.A., T.A. Wani, N.Y. Khalil, A. Al-Shaikh, N. Al-Morshadi, (2012). Development of a novel 96-microwell assay with high throughput for determination of olmesartan medoxomil in its tablets. Chemistry Central Journal, 6(1): 1-7.
23. Darwish, I.A., T.A. Wani, N.Y. Khalil and A.H. Backeit, 2012. Novel 96-microwell spectrophotometric assays with high throughput for determination of irbesartan in its tablets. Digest Journal of Nanomaterials and Biostructures, 7(2): 415-421.
24. Fidler, A.T., E.L. Baker, R.E. Letz, 1987. Neurobehavioural effects of occupational exposure to organic solvents among construction painters. British Journal of Industrial Medicine, 44(5): 292-308.
25. Kristensen, P., B. Hilt, K. Svendsen and T.K. Grimsrud, 2008. Incidence of lymphohaematopoietic cancer at university laboratory: a cluster investigation. European Journal of Epidemiology, 23(1): 11-15.
26. Lindbohm, M.L., H.T. Taskinen, M. Sallman and K. Hemminki:, 2007. Spontaneous abortions among women exposed to organic solvents. American Journal of Industrial Medicine, 17(4): 449-463.
27. Wennborg, H., J.P. Bonde, M. Stenbeck and J. Olsen, 2002. Adverse reproduction outcomes among employee working in biomed-ical research laboratories. Scandinavian Journal Work, Environment and Health, 28(1): 5-11.
28. Wennborg, H., B. Lennart, V. Harri and A. Gösta, 2000. Pregnancy outcome of personnel in swedish biomedical research laboratories. Journal of Occupational and Environmental Medicine, 42(4): 438-446.
29. ICH Guideline, Q2(R1), 2005. Validation of analytical procedures: text and methodology. London.
30. Champix®, Consumer medicine information, published by MIMS/myDr, September 2013. http://www.mydr.com.au/cmis/ReducedPDFs/CMR09040.pdf. Browsing date: Oct. 25, 2013.