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Validation of Spectrophotometric Method to Quantify Veranicline Content in Tablets

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Varenicline is a nicotinic receptor partial agonist used to treat smoking addiction. The objective of this work was to develope and validate UV-Vis spectrophotometric method for the determination of veranicline in tablets. In this study, 0.01 M phosphate buffer arranged to pH 7 was used to prepare standard stock solutions from varenicline tartrate salt, as well used to dissolve the commercials tablet and synthetic tablet solutions. UV-VIS spectrophotometric determination was performed at 319 nm wavelength having no interference coming from matrix components. The developed method was linear within the range 1-100 μg mL^{-1}. Method validation was performed according to the ICH guideline and the results show that this simple and low cost method is precise, accurate, robust and rugged to be proposed for the routine analysis in quality control laboratories.

Key Words: Varenicline tartrate, Spectroscopy, Determination, Method validation, Pharmaceutical formulation.

INTRODUCTION

Drug dependence is a chronic brain disease characterized by recurrent episodes of relapse, even when the person is motivated to quit. The nicotinic acetylcholine receptor (nAChR) plays an important role in nicotine dependence, alcohol consumption and cueinduced cocaine craving. Stimulation of the nicotinic acetylcholine receptor has been found to alter and modulate cell firing in brain areas important for the maintenance of drug dependence. Varenicline is an α4β2 nicotinic acetylcholine receptor partial agonist and an α7 nicotinic acetylcholine receptor full agonist registered for the treatment of nicotine dependence, significantly reduces nicotine craving and prevents relapse[1]. Varenicline, as the tartrate salt, is a powder, which is a white to off-white to slightly yellow solid with the following chemical name: 7,8,9,10-tetrahydro-6,10-methano-6*H*-pyrazino[2,3- h][3]benzazepine, (2R, 3R)-2,3-dihydroxybutanedioate (1:1). It is highly soluble in water. Varenicline tartrate has a molecular weight of 361.35 daltons and a molecular formula of $C_{13}H_{13}N_3 \cdot C_4H_6O_6$[2]. The chemical structure of varenicline tartrate is given in Fig. 1. Analysis of active drug compounds in pharmaceutical formulations are the routine process in quality control laboratories and it is important to use precise and accurate analytical techniques in order to perform these quality control process. Therefore, validation of an analytical method is the key point to propose a newly developed technique to be used in the routine of the quality control. In the literature, it is reported that determination of varenicline in tablets has already been performed by HPLC and UPLC techniques[3-5]. However, an UV-VIS spectrophotometric method has not been proposed yet to perform analysis. It is well known that UV-VIS spectrophotometric techniques are easy to apply, simple and low cost methods in comparison to other analytical methods such like HPLC and capillary electrophoresis. Thus, it is decided to develope and validate an UV spectrophotometric method for the analysis of varenicline in pharmaceutical formulations. In this study, it is discussed the optimum conditions, the tablet analysis procedure, the linearity range and the sensitivity of the proposed method. According to the ICH quideline, the method is fully validated[6].

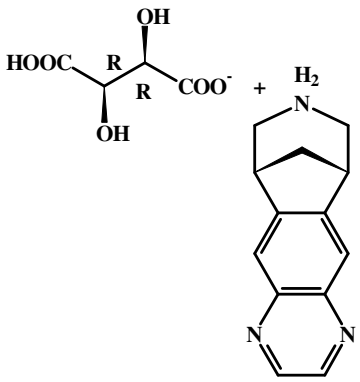

Fig. 1. Chemical structure of varenicline tartrate

EXPERIMENTAL

The spectrophotometric measurments were carried out using an Aglient 8453 model UV-VIS spectrophotometer with a diode array detector (DAD) (190-1100 nm). UV spectra of reference and sample solutions were recorded in 1 cm quartz cells. The detector was set at 319 nm wavelength.

Standard varenicline tartrate supplied by Refik Saydam National Public Health Agency. $NaH_2PO_4 \cdot 2H_2O$ and NaOH were from Merck (Darmstadt, Germany). Water obtained from the Milli-Q water system (Barnstead, USA) was used for the preparation of buffer. Commercially available tablets (CHAMPIX®) containing 1 mg of varenicline (as equal to 1.71 mg of varenicline Tartrate)/0.5 mg of varenicline (as equal to 0.85 mg of varenicline Tartrate) were purchased from a local pharmacy.

Phosphate buffer solution (0.01 M, pH 7): 1.56 g of $NaH_2PO_4 \cdot 2H_2O$ was dissolved in 900 mL of water and the pH was adjusted to 7 by adding 1 M NaOH solution. Total volume was filled up to 1000 mL by adding water.

Standard stock solution (1000 μg mL^{-1}): 25 mg of varenicline tartrate was dissolved in 25 mL of phosphate buffer solution.

Calibration standards: Calibration standards of veranicline tartrate (1.0, 5.0, 10.0, 20.0, 50.0, 80.0 and 100.0 μg mL^{-1}) were daily prepared from standard stock solution by appropriate dilution processes using phosphate buffer solution.

Tablet solutions: Ten tablets were weighed to determine their mean weight and finely powdered in a mortar. An amount of powdered mass equivalent to one tablet was accurately weighed and transferred to a 25 mL volumetric flask. 20 mL Volume of phosphate buffer was added then sonicated for 15 min to ensure complete extraction of the drugs. The flask was then filled up to volume with phosphate buffer. An aliquot from this solution was filtered through a 0.45 μm membrane filter. The final tablet solutions contain 20 and 40 μg mL^{-1} of varenicline depending to the pharmaceutical dosage forms (CHAMPIX® tablets contain 0.5 mg and 1 mg of varenicline). These amounts are equal to 34.2 and 68.4 μg mL^{-1} of varenicline tartrarte, respectively.

Placebo solutions: According to the literature, the reported ingredients in the formulation were already known[2]. Microcrystalline cellulose (10 %, 100 mg), anhydrous dibasic calcium phosphate (83 %, 830 mg), croscarmellose sodium (5 %, 50 mg), colloidal silicon dioxide (1 %, 10 mg) and magnesium stearate (1 %, 10 mg) were weighed to achieve 1 g of bulk. Then, approximately 0.1 and 0.2 g of this bulk were used to prepare the placebo solutions for the two different dosage forms (tablets containing 0.5 mg and 1 mg of varenicline). Placebo solutions were prepared same as preparation of tablet solutions. Sythetic tablet solutions were prepared by adding known amounts of varenicline standard solutions to varenicline placebo solutions.

RESULTS AND DISCUSSION

Varenicline, as the tartrate salt, is a white to slightly yellow powder and highly soluble in water[7]. The water solubility of varenicline tartrate make it possible to perform the analysis and extract the varenicline in tablets through aqueus solutions. In order to realise whether the pH of the aqueus solutions would shift the λ_{max} value and change the formation and shape of the spectrum, various solutions such 0.1 M HCl, 0.1 M NaOH and 0.01 M phosphate buffer (pH 7) were experienced to perform the analysis. It was obvious that the pH of the solution was changing both the formation of the spectrum and local λ_{max} values (Fig. 2). Therefore, it was necessary to use a buffer solution to keep the pH stable. The peaks and the valleys for acidic, basic and neutral pHs are given in Table-1.

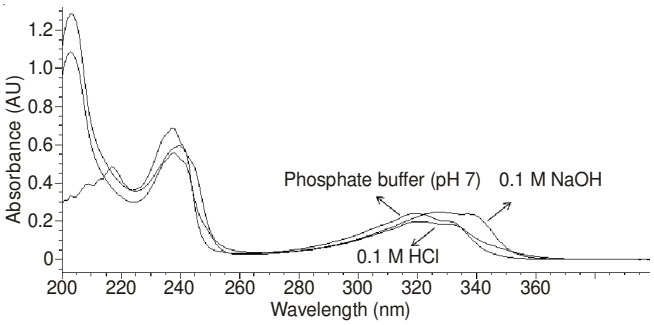

Fig. 2. UV spectra of varenicline in acidic, basic and neutral conditions

TABLE-1
LINEARITY DATA OF THE DEVELOPED METHOD (n=6)

	Varenicline tartrate
Regression equation*	y = 0.0232 x + 0.0037
Standard error of intercept	0.0019
Standard error of slope	0.0001
Correlation coefficient (r)	0,9998
Calibration curve (μg mL^{-1})	1.0-100.0
Number of data points	7
LOD (μg mL^{-1})	0.004
LOQ (μg mL^{-1})	0.013

*where y is absorbance and x is concentration in μg mL^{-1}

Using an organic solvent was another option to develope the method. Since, the idea at the begining was to develope a simple, rapid, valid and low cost method against the HPLC methods, it was avoided organic solvents and only used aqueus solutions to construct the calibration curves, as well perform the tablet analysis. Using 0.01 M phosphate buffer adjusted to pH 7 was able to perform the analysis properly. Thus, it was used phosphate buffer not having any interference to varenicline spectrum.

The proposed method was validated by evaluating the stability, linearity range, sensitivity, precision, accuracy, specificity/selectivity, robustness and ruggedness according to the ICH guideline[6].

Stability: Stability of varenicline is already known through previous studies and it is clear that varenicline is stable at water even at ambiant temperature (25 ± 1 ºC) for over 3 days[4]. However, in this study, the standard stock solutions of varenicline was prepared in phosphate buffer (0.01 M, pH 7) and stored at - 4 ºC for one week while being prevented from daylight. During this period, UV spectrum of the solutions were taken periodically. They were compared with freshly prepared solutions and no statistical difference was found between them when it was applied Wilcoxon signed-rank test ($p < 0.05$). This indicated that varenicline was stable under the above mentioned conditions at least for one week.

Linearity range and sensitivity: In quantitative analysis the calibration curve was constructed (Fig. 3) after analysis of consecutively increased concentrations (1.0, 5.0, 10.0, 20.0, 50.0, 80.0 and 100.0 μg mL^{-1}). The regression equation, standard errors of slope and intercept, correlation coefficient and linearity range were given in Table-1. The limit of detection (LOD) (k = 3.3) and limit of quantition (LOQ) (k = 10) of the method were established according to the ICH definitions (C1 = k S_0/s where C1 is LOD or LOQ S_0 is the standard error of blank determination, s is the slope of standard curve and k is the constant related to the confidence interval).

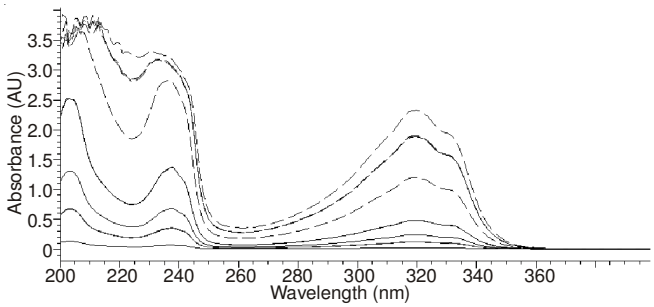

Fig. 3. Varenicline tartrate spectra on various concentrations (1.0, 5.0, 10.0, 20.0, 50.0, 80.0 and 100.0 μg mL^{-1}) at pH 7 phosphate buffer

Precision and accuracy: Various levels of drug concentrations were prepared from standard stock solution and analyzed. Interday and intraday variations were studied to determine intermediate precision of the proposed analytical method. The per cent differences between the measured mean concentrations and the corresponding nominal concentrations were calculated to indicate the accuracy of the method. Different levels of drug concentrations in triplicates were prepared six different times in a day and studied for intraday variation. The same procedure was followed for six different days to study interday variation (n = 10). The per cent relative standard deviation (R.S.D. %) of the predicted concentrations and (Table-2).

TABLE-2
PRECISION AND ACCURACY OF THE DEVELOPED METHOD

Added (μg mL^{-1})	Intra-day		
	Found (μg mL^{-1})	Precision RSD (%)	Accuracy bias (%)
5	4.97 ± 0.13	2.55	-0.68
20	20.27 ± 0.40	1.98	1.34
80	81.69 ± 1.64	2.01	2.11
	Inter-day		
5	4.88 ± 0.09	1.86	-2.41
20	20.19 ± 0.49	2.44	0.89
80	80.30 ± 0.86	0.94	0.38

Found: mean ± standard error (n=6); RSD : Relative standard deviation; Bias : [(Found - Added) / Added] × 100]

Recovery experiments were conducted to determine the accuracy of the proposed method. Standard addition technique was applied to the same preparations which were analyzed by the calibration curve method. The standard addition technique (often referred to as spiking the sample) is commonly used to determine the concentration of an analyte that is in a complex matrix such as biological fluids, soil samples, *etc*. The reason for using the standard additions method is that the matrix may contain other components that interfere with the analyte signal causing inaccuracy in the determined concentration. The idea is to add analyte to the sample (spike the sample) and monitor the change in instrument response. The change in instrument response between the sample and the spiked samples is assumed to be due to change in analyte concentration[8]. The regression equations of standard addition curves of the method for tablet analysis were found to be y = 0.0233 ± 0.0004 x + 0.7913 ± 0.0176. Since the slopes of the standard calibration and standard addition curves were identical, it was concluded that there was no spectral interaction in the analysis of varenicline in tablet dosage forms with the developed method.

Another recovery study was performed by adding known amounts of varenicline tartrate standard solutions to the placebo solutions in order to achieve 34.2 and 64.8 μg mL^{-1} concentrations. These concentrations were identical in real tablet solutions. Six samples were prepared for each recovery level. The mean value of the recovery for the developed method was 99.69 ± 0.70 (mean ± standard error), which is inside the satisfactory range clearly[9,10].

Specificity/Selectivity: The USP monograph defines the selectivity of an analytical method as its ability to measure accurately an analyte in the presence of interference, such as synthetic precursors, excipients, enantiomers and known (or likely) degradation products that may be expected to be present in the sample matrix[11]. Comparison of the UV spectra of standard and tablet solutions showed that the wavelenght of maximum absorbance did not change and the spectra obtained from standard, tablet and synthetic tablet solutions containing an equivalent concentration of varenicline tartrate were identical for the developed method (Fig. 4). It could be concluded that excpients did not interfere with quantitation of varenicline in this method.

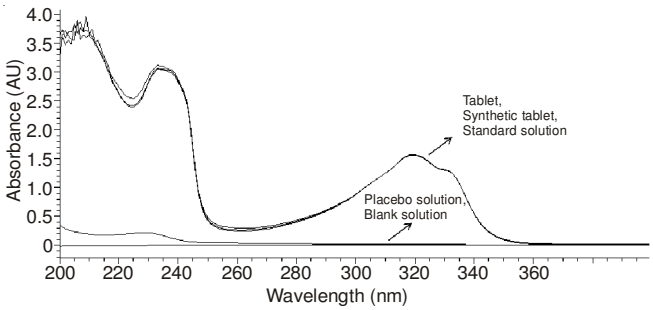

Fig. 4. Spectra of placebo, blank (0.01 M phosphate buffer pH 7), tablet, synthetic tablet and standard solutions. Tablet, synthetic tablet and standard solutions contain 68.4 μg mL^{-1} of varenicline tartrate

Robustness and ruggedness: The robustness of the proposed method was tested by changing parameters such as wavelength range (± 1 nm) and buffer pH (± 0.1). None of these variables significantly affected the absorbance of varenicline. The ruggedness of the developed method was shown by applying same procedures by two different operators. The analysis results having no significant difference indicate that the proposed method is robust.

Tablet analysis: The developed method was successfully applied to the determination of varenicline in its pharmaceutical

tablet formulations (CHAMPIX® tablets contain 0.5 mg and 1 mg of varenicline). Varenicline tartrate in six different tablet solutions derived from two dosage forms were analyzed by calibration curve technique. Analysis results are given in Table-3. These results indicate that the proposed method could be succesfully applied in routine analysis of varenicline in tablets.

TABLE-3
VARENICLINE ANALYSIS IN TABLET DOSAGE FORMS

Tablet solutions	CHAMPIX® tablets (1.0 mg and 0.5 mg Varenicline)	
	1 mg	0.5 mg
1	0.98	0.52
2	0.98	0.52
3	1.02	0.51
4	0.98	0.51
5	1.00	0.49
6	1.00	0.49
Mean ± SE	0.99 ± 0.02	0.51 ± 0.01
RSD	1.51	2.01
Bias	-0.53	1.40

Found : mean ± standard error (n=6); RSD : Relative standard deviation; Bias : [(Found - Added) / Added] x 100

Conclusion

The method was found to be simple, accurate, precise, rugged and robust. It can be directly and easily applied to the analysis of the pharmaceutical tablet formulation of varenicline. Moreover, the present method is fast and inexpensive incomparison to chromatographic techniques. Therefore, it can be concluded that the proposed method provides an alternative and simple procedure for the quality control of varenicline in pharmaceutical formulations.

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