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Development and validation of the UV spectroscopic method for varenicline determination in pharmaceutical preparation

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Abstract--A simple and accurate UV visible spectroscopic method has been developed and validated for quantification of varenicline in bulk drug and pharmaceutical dosage forms. A shimadzu UV spectrophotometer was used considering availability and cost effectiveness of the proposed method. λ_{max} of 236nm and 319nm were observed from the spectroscopic method. The calibration curve was linear ($r = 0.99$) in the studied range of concentration (5- 40 $\mu\text{g} / \text{ml}$) in both the absorption maxima. The selectivity and sensitivity of the elaborated method were satisfactory, and the limits of detection and quantification were less than 20% of the specification level. Moreover, the inter- and intra-day precisions was found to be less than 3% (RSD), while the recovery values expressing inter- and intra-day accuracy was varied from 99.73 to 101.23. The varenicline solution was stable over a period of 3 days on storage under refrigeration. The utility of the developed method was examined by analyzing the tablets containing VAR. As a result, the method was found to be selective, sensitive, precise and accurate.

Keywords--UV visible spectroscopic method, varenicline, smoking.

Introduction

Smoking is the number one cause of preventable disease and death in the United States (Young & Davis,2019). A number of smoking cessation pharmacotherapies

have led to rises in quitting and thus to important benefits to public health (Pietras et al., 2017). There are now various FDA-approved medications that are effective in helping individuals quit smoking, including nicotine replacement therapy (NRT), bupropion sustained release treatment and varenicline. It was found in a large meta-analysis varenicline is to be the most effective monotherapy available and nicotine replacement, the most commonly used medication for smoking cessation (Hajek, Smith, Dhanji, & McRobbie, 2013).

Varenicline is a novel and a potent and selective partial agonist for $\alpha 4\beta 2$ nicotinic acetylcholine receptors, has been used for smoking cessation treatment (Kwak et al., 2018). Both nicotine and varenicline bind to this receptor subtype. Varenicline blocks the ability of nicotine to activate $\alpha 4\beta 2$ receptors and this to stimulate the central nervous mesolimbic dopamine system, believed to be the neuronal mechanism underlying reinforcement and reward experience upon smoking (Kadi, Mohamed, Kassem, & Darwish, 2011). Varenicline is a partial agonist at $\alpha 4\beta 2$ neuronal nicotinic acetylcholine receptors where it binds with high affinity and selectivity to produce an effect sufficient to alleviate symptoms of craving and withdrawal (agonist activity), while simultaneously resulting in blockade of the rewarding and reinforcing effects of smoking by preventing nicotine binding to $\alpha 4\beta 2$ receptors (antagonist activity). Literature depicted that varenicline showed significant benefit over NRT in measures of craving and withdrawal by decreasing the urge to smoke, negative effect and restlessness (Koegelenberg et al., 2014). VAR tartrate (Champix[®] and Chantix[®]: Pfizer) has been approved by USFDA as an aid of smoking cessation (Zierler-Brown & Kyle, 2007). The approved regime of VRC is 1mg for 12 weeks, starting with 1-week titration period.

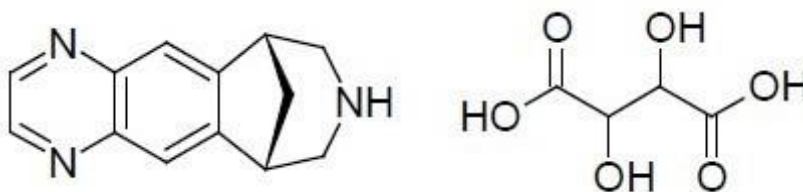


Figure 1. Structure of Varenicline Tartrate (VAR)

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-6H-pyrazino [2,3- h] benzazepine, (2R,3R)- 2,3-dihydroxybutanedioate (1:1) (Hajek et al., 2013). It is highly soluble in water. Varenicline tartrate has a molecular weight of 361.35 Daltons and a molecular formula of $C_{13}H_{13}N_3 \cdot C_4H_6O_6$. The chemical structure of varenicline tartrate is given in Figure 1.

Analysis of active drug compounds in pharmaceutical formulations is the routine process in quality control laboratories and it is important to use precise and accurate analytical techniques in order to perform this 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 varenicline tartrate in tablet has already been performed by HPLC and UPLC techniques. However, an UV-VIS spectrophotometric method of analysis is simple, easy to apply and low cost method in compare to other analytical method. Quality control of varenicline-containing preparations using a simple, sensitive, precise and accurate UV method is necessary due to the fact that these preparations are often imported and are often exposed to different transport conditions(Carpenter et al., 2013). The present study describes the development and validation of the UV method for the quantitative determination of VAR in its pharmaceutical preparation.

Materials

Varenicline Tartrate standard substance was purchased from Sigma-Aldrich (Steinheim, Germany). Champix® tablets (Pfizer, New York, USA), labelled to contain 1 mg (as the anhydrous base) per tablet was obtained from the local market. Trifluoroacetic acid for spectroscopy (Uvasol®) and hexane-1- sulphonic acid sodium salt came from Merck (Darmstadt, Germany), hydrogen peroxide solution 30% - from Chempur (Piekary Slaskie, Poland), magnesium stearate - from Acros Organics-Thermo Fisher Scientific (Geel, Belgium). Sodium benzoate was obtained from Sigma-Aldrich (Steinheim, Germany), while HPLC grade solvents and all other chemicals were procured from POCh (Gliwice, Poland). The water used throughout the study was double distilled.

Preparation VAR stock solution

The stock solution of VAR at a concentration of 1 mg/ml was prepared by dissolving 10 mg of the substance to be examined in 10.0 ml of water.

Stress testing

To obtain the VAR working solution (10 µg/ ml), 1 ml of the VAR stock solution was diluted to 100 ml with water.

Acid hydrolysis, alkali hydrolysis, oxidation

An aliquot of 1 ml of the VAR working solution was transferred to a vial and mixed with 1 ml of the appropriate solution: 1 mol/l solution of hydrochloric acid, or 2 mol/l solution of sodium hydroxide, or 30% solution of hydrogen peroxide. The vials were placed in a water bath at 80°C for 4 h, then cooled to room temperature and the concentration was measured using UV spectrophotometer.

Irradiation with UV light

An aliquot of 1 ml of the VAR working solution was transferred to a quartz cuvette and exposed to UV light at wavelength of 245 nm (10 W/m^2) for 4 hours, and then the concentration was measured using UV spectrophotometer.

Preparation of calibration solutions

Calibration solutions at concentrations of 5, 10, 15, 20, 25, 30, 35 and 40 $\mu\text{g}/\text{ml}$ were prepared by the appropriate diluting of the stock solution of VAR with water and subjecting it to spectrophotometric analysis.

Accuracy

Herein, 10 tablets were weighed and the average weight was calculated. The tablets were then crushed to a fine powder, and a quantity of the powdered tablets, equivalent to 0.5 mg of VAR, was transferred to 10 ml volumetric flasks. Subsequently, 5 ml of water was added, the contents of the flasks were shaken for 5 min, the volume was diluted to the mark with water (50 $\mu\text{g}/\text{ml}$) and accurately mixed. Next, the contents of the flasks were transferred to centrifuge tubes and centrifuged at 10 000 rpm for 5 min, then an aliquot of 0.2 ml of clear supernatant was taken from each tubes, transferred to 10 ml volumetric flasks, diluted to the mark with water (1.0 $\mu\text{g}/\text{ml}$) and evaluated with UV spectrophotometer. This procedure was repeated six times in the same day and twelve times for two days.

Preparation of tablet model mixtures for Recovery

Tablet model mixtures (M50%, M100%, M150%) were prepared by adding 50%, 100% and 150% of VAR standard in relation to the label claim, to a placebo matrix containing calcium hydrogen phosphate, magnesium stearate, zinc oxide and starch. The extraction of VAR from the tablet model mixtures was performed in the same manner as for tablet samples. The procedure was repeated three times for each tablet model mixture.

Result and Discussion

Method development

This work presents the validation of the UV VIS spectrophotometric method developed to examine varenicline content in the selected pharmaceutical formulation. For the chromatographic analysis, a Shimadzu UV spectrophotometer was used. The initial method development was conducted on pure substance using working standards solution. Although the light absorption characteristics of VAR are available in literature (Jiloha, 2010; Silagy, Lancaster, Stead, Mant, & Fowler, 2004; Wilkes, 2008; Zatonski, Cedzynska, Tutka, & West, 2006), we recorded the UV-absorption spectrum in methanol in order to select the appropriate analytical wavelength, while simultaneously verifying available data. As shown in Figure 1, VAR has two absorption maxima at 236 and 319 nm which were considered in further analysis. No significant differences between the spectrum in methanol and the spectra available in literature were observed.

System suitability

We tested the system's suitability for being an integral part of an analytical procedure. This was done with respect to the signal to-noise ratio for the VAR peak using a VAR solution of 10 µg/ml. The high value of signal-to-noise ratio of 2.00E+05 - indicates the use of a suitable analytical wavelength. All these results assure the adequacy of the proposed UV spectroscopic method for the routine analysis of VAR.

Sample solution stability

The stability of the drug in solution during analysis was determined by repeated analysis of drug samples (10 µg/ml) during the same day and also after storage of the drug solution for three days under refrigeration. The results of such experiments indicate that there was no significant change in analysis over a period of 72 hours. Herein, the mean RSD between peak areas for the samples was found to be less than 0.5%, suggesting that the drug solution can be stored without any degradation over the time interval studied.

Stress testing of VAR

Forced degradation studies was carried out to demonstrate the selectivity and stability-indicating property of the proposed method. In so-doing, stress testing of drug substances can detect the changes with time in the properties of the drug substance. In our experiment, different stress conditions were applied, among these, acid and base hydrolysis, oxidation and irradiation with UV light. In doing this, VAR was treated with 30% solution of hydrogen peroxide, 1 mol/l solution of hydrochloric acid, 2 mol/l solution of sodium hydroxide and UV radiation (254nm). The applied conditions resulted in the significant decomposition of the analysed substance, and λ max of the degradation products were different from those of VAR. In the case of oxidation, absorption was observed at λ max similar to that of VAR was observed, but with a significantly larger area.

Linearity

The ability to obtain detector signals proportional to the concentrations of VAR in the samples was tested by the construction of five independent calibration curves. Each curve was generated by creating 6-concentration points; each concentration was analysed in triplicate, and regression analysis for the results was carried out using the least-square method. The results revealed a good linear calibration fit in the range of 5-40 µg /ml. The calibration equation is shown in the figure 2 with the determination coefficient of 0.9935 and 0.9964 for λ max value of 236 and 319 nm. Linearity was confirmed by significance testing of Pearson correlation ($TV = 539.7 (28) > t = 1.71$) and by Mandel fitting test ($TV = 0.433 < F_{99\%} (1, 27) = 7.68$). The obtained low values of standard deviations of the regression coefficients are indicative for the significant validity of the calibration points used for constructing the calibration curve.

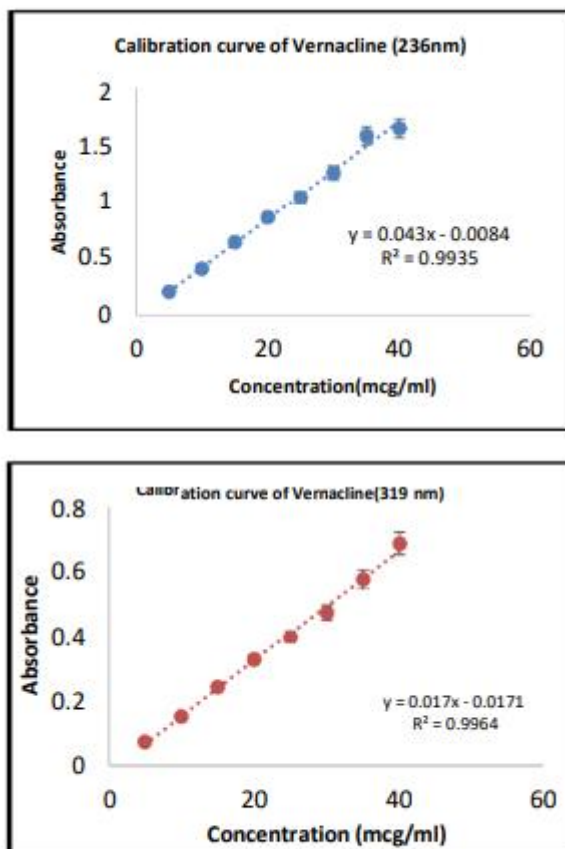


Figure 2. Calibration curve of VAR at 236nm and 319nm

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the signal-to-noise method (Pujeri, Khader, & Seetharamappa, 2012). Determination of the signal-to-noise ratio was performed by comparing measured signals from samples with known low concentrations of VAR, with those of blank samples, and then establishing the minimum concentration at which the VAR can be reliably detected. A signal-to-noise ratio of 3:1 or 10:1 was taken into account for estimating the LOD and LOQ, respectively. The LOD and LOQ values were 0.82 and 1.33 $\mu\text{g}/\text{ml}$ respectively.

Precision

Precision was measured in accordance with ICH recommendation. Six solutions of VAR at three different concentrations (9, 3, and 0.3 $\mu\text{g}/\text{ml}$) showed acceptable intra-day precision; the relative standard deviations (RSD, $n = 3$) of the concentrations, calculated from a calibration curve, did not exceed 3%. Inter-day precision was determined by multiple inter-day measurements of VAR at the

same VAR concentration level (9, 3, and 0.3 µg/ml). The RSD values (n = 3) of less than 3% indicate acceptable reproducibility of the method (Table 1).

Table 1. Precision of proposed method

| The concentration(mcg/ml) | labelled | The found concentration(mcg/ml) | RSD(%) |
|---------------------------|----------|---------------------------------|--------|
| 9 | | 9.009 | 0.54 |
| 3 | | 3.009 | 0.67 |
| 0.3 | | 0.297 | 2.59 |
| Interday Precision | | | |
| 9 | | 8.967 | 0.45 |
| 3 | | 3.008 | 0.69 |
| 0.3 | | 0.297 | 3.08 |

Accuracy

Accuracy was based on the recovery study of known amounts of VAR standard added (50%, 100% and 150% in relation to the label claim) to a placebo matrix for tablets (tablet model mixtures). The samples at each concentration level were injected in triplicate, and the added amounts were calculated from a calibration curve. The recovery was expressed as percentages, calculated from the formula: concentration/nominal concentration × 100). The recovery values ranged from 99.73 to 101.23 (Table 2). The obtained results indicate acceptable method accuracy.

Table 2. Evaluation of the method accuracy by recovery study

| Tablet model mixture | The labelled concentration(mcg/ml) | The found concentration (mcg/ml) | % Recovery |
|----------------------|------------------------------------|----------------------------------|------------|
| M 150% | 1.85 | 1.873 | 0.54 |
| M 100% | 1.25 | 1.247 | 0.67 |
| M 50% | 0.6 | 0.598 | 2.59 |

*mean value, n=3.

Applicability of the method

As shown above, the developed method gave satisfactory results with the analysis of VAR in bulk substance. Thus, VAR-containing tablets were subjected to analysis by the proposed method. In such an experiment, the label claim percentage was found to be $99.96 \pm 1.31\%$ RSD (intra-day analysis) and $99.46 \pm 1.25\%$ RSD (inter-day analysis). These satisfactory values indicate that the proposed method can be applied for the routine quality control of VAR tablets (Table 3).

Table 3. Spectroscopic Characteristic

| Sr. No. | Parameters | Results |
|---------|---|----------------------------|
| 1 | λ_{max} | 236nm, 319 nm |
| 2 | Beer's law limit, $\mu\text{g/mL}$ | 05 to 40 |
| 3 | Regression equation | $y = 0.043x - 0.0084$ = |
| 4 | Correlation coefficient (r^2) | 0.9935 0.9964 |
| 5 | Limit of Detection (LOD), $\mu\text{g/mL}$ | 0.82 |
| 6 | Limit of Quantitation (LOQ), $\mu\text{g/mL}$ | 1.33 |
| 7 | Intraday precision (% R.S.D) | 1.31 |
| 8 | Interday precision (% R.S.D) | 1.25 |

Conclusion

The developed UV VIS spectrophotometric method is selective, sensitive, accurate and precise. Statistical analysis for the results demonstrates that the method is suitable for the determination of VAR in bulk and tablet forms without the interference generated via degradation products. It is, therefore, recommended for routine use in laboratory quality control.

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