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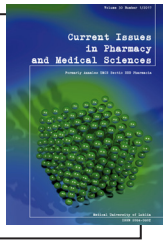

Development and validation of the HPLC method for varenicline determination in pharmaceutical preparation

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ABSTRACT

A simple and accurate reverse phase HPLC method has been developed and validated for quantification of varenicline in bulk drug and pharmaceutical dosage forms. Herein, an isocratic LC analysis was carried out on a Chromolith Performance RP18e column with methanol-buffer solution pH 3.5 (a buffer solution containing sodium benzoate (0.5 mmol/l) adjusted to pH 3.5 with trifluoroacetic acid (20 mmol/l) (55:45, *V/V*)) at a flow rate of 1.2 ml/min. The detection wavelength was set at 320 nm. The calibration curve was linear (r = 0.9999) in the studied range of concentration (0.2-10 μg/ml). The selectivity and sensitivity of the elaborated method were satisfactory, and the limits of detection and quantification was 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 analysing the tablets containing VAR. As a result, the method was found to be selective, sensitive, precise and accurate.

INTRODUCTION

A number of smoking cessation pharmacotherapies have led to increases in quitting and thus to significant benefits to public health [1]. One of the strategies for smoking cessation is nicotine replacement therapy (NRT). NRT aims to reduce motivation to consume tobacco and the physiological and psychomotor withdrawal symptoms through delivery of nicotine without the dangerous chemicals found in cigarettes [2]. Nicotine replacement products take a number of forms, such as chewing gum, oral inhaler, nasal spray, transdermal patch and tablet. In this type of therapy, the proper application of nicotine containing medications is crucial. Nicotine stimulates the acetylcholine receptors (traditionally classified as nicotine receptors) concentrated in the midbrain areas. In addition, it binds to the receptors in nigrostriatal and mesolimbic dopaminergic neurons. Nicotine is one of the most potent stimulants of the midbrain dopamine reward pathway [3].

Until recently, NRT has been the only recognised form of treatment for smoking cessation. Other forms of treatment have, however, now come onto the market. Antidepressant-bupropion is the first non-nicotine based drug which was introduced for smoking cessation. Bupropion exerts its effect primarily through the inhibition of dopamine re-uptake into neuronal synaptic vesicles [4]. The increase of the concentration of this neurotransmitter produces feelings similar to those after cigarette smoking. Bupropion leads to the reduction of tobacco craving, as well as to the reduction of the symptoms of withdrawal syndrome. Cytisine, a plant-derived alkaloid, is also now available in the treatment of nicotine dependence. Cytisine, like varenicline, is structurally similar to nicotine and acts as a partial agonist of nicotinic acetylcholine receptors (nAChR) as it has a high affinity for the alpha4beta2 receptor subtype. With regard to cytisine, the long-term abstinence rates were similar to those observed in smokers receiving nicotine replacement therapy [5].

Varenicline; 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)benzazepine (VAR, Figure 1) is a relatively novel agent that is a centrally acting as a highly selective partial agonist of the mentioned alpha4beta2 nAChR subtype. This activity at the nicotine-receptor level may help patients achieve smoking cessation by reducing cravings/withdrawal symptoms and by providing smoking satisfaction. Available clinical-trial data support its use as an effective and generally well-tolerated therapy for smoking

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cessation in healthy adult smokers [6]. High performance liquid chromatography (HPLC) is the predominant method used for varenicline analysis [7-14]. Other analytical techniques are used sporadically, including mainly spectroscopic techniques [10,15,16]. Varenicline has been analyzed using UV-VIS spectrophotometry [15], fluorimetry [16], nuclear magnetic resonance spectroscopy (1H NMR and 13C NMR) [10], mass spectrometry (MS) [10,13,14] and Fourier-transform infrared spectroscopy (FTIR) [10]. Most frequently, papers refer to the quantification of varenicline in its pharmaceutical preparations [7-12,15,16].

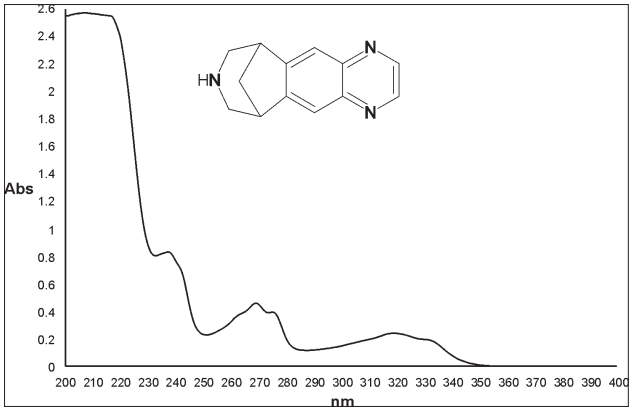

Figure 1. Chemical structure of VAR and its absorption spectrum (concentration of VAR was 10 μg/ml in methanol)

Quality control of varenicline-containing preparations using a simple, sensitive, precise and accurate HPLC methods is necessary due to the fact that these preparations are often imported and are often exposed to different transport conditions [9].

The present study describes the development and validation of the HPLC method for the quantitative determination of VAR in its pharmaceutical preparation. The advantage of the proposed liquid chromatographic procedure was the use of a Chromolith Performance column for VAR analysis. Beyond this, the addition of benzoic acid as ion pairing agent made it possible to analyse VAR at a wavelength of 320 nm.

MATERIAL AND METHODS

Materials

Varenicline Tartrate standard substance was purchased from Sigma-Aldrich (Steinheim, Germany). Champix® tablets (Pfizer, New York, USA), labeled 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.

Chromatographic system

All HPLC measurements were made on a Waters Alliance HPLC system (Waters, USA) equipped with a 2998 photodiode array detector, with data processing on Empower 2.0 version software. The chromatographic analysis was performed on a Chromolith Performance RP18e fully end-capped column (100 × 4.6 mm, macropores 2 μm, mesopores 13 nm) from Merck. The column temperature was kept constant at 22.5±1°C. Separations were performed in the isocratic mode, using a mobile phase consisting of methanol and buffer solution containing sodium benzoate (0.5 mmol/l) adjusted to pH 3.5 with trifluoroacetic acid (20 mmol/l) (55:45, *V/V*) and degassed by ultrasonic bath. The flow rate of the mobile phase was 1.2 ml/min, and the sample injection volume was 20 μl, while the PDA detector was set at 320 nm. The UV spectra were obtained using spectrophotometer UV-VIS U2001 (Hitachi Instruments, USA).

Preparation VAR stock solution

The stock solution of VAR at a concentration of 0.1 mg/ml was prepared by dissolving 1 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 10 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 80oC for 4 h, then cooled to room temperature and injected into the HPLC system.

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 injected into the HPLC system.

Preparation of calibration solutions

Calibration solutions at concentrations of 0.2, 0.5, 1, 2, 5 and 10 μg/ml were prepared by the appropriate diluting of the stock solution of VAR with water and subjecting it to chromatographic analysis.

Preparation of tablet samples

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 mechanically, the volume was then diluted to the mark with water (50 μg/ml) and accurately mixed. Next, the contents of the flasks was 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 μg/ml) and injected into the HPLC

system. This procedure was repeated six times in the same day and twelve times for two days.

Preparation of tablet model mixtures

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.

RESULTS AND DISCUSSION

Method development. This work presents the validation of the high performance liquid chromatographic method with UV-detection developed to examine varenicline content in the selected pharmaceutical formulation. For the chromatographic analysis, a Chromolith HPLC column was chosen. This type of column consists of a single rod of high purity monolithic silica and has a porosity exceeding 80%, and a unique bimodal pore structure; consequently, these columns give greatly improved chromatographic performance.

The initial method development was conducted on pure substance using working standards solution. Although the light absorption characteristics of VAR are available in literature [9,12], 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 three absorption maxima at 237, 269 and 320 nm which were considered in further analysis. No significant differences between the spectrum in methanol and the spectra available in literature were observed. For the VAR analysis, the methanol spectrum was chosen because its mobile phase contained 55 percent of methanol. In the experiment, the chromatographic conditions were established after a number of preliminary experiments undertaken so as to select the pertinent mobile system. The composition of the mobile phase systems were tested taking into account the effect of acetonitrile or methanol and the buffered mobile phases containing phosphate, citrate or acetate buffer at different pHs on the varenicline retention and symmetry of the varenicline peak. Acceptable results were obtained using methanol as the modifier, but the investigated buffered mobile phases did not give the expected changes of the chromatographic system parameters. Therefore, the influence of two ion-pairing agents, hexane-sulphonic acid sodium salt and sodium benzoate, on the retention of varenicline was explored. Herein, the use of a mobile phase containing sodium benzoate adjusted to pH 3.5 with trifluoroacetic acid gave satisfactory chromatographic performance: a symmetrical varenicline peak with a low retention time. Moreover, the formation of a varenicline-benzoate ion pair intensified the absorption of VAR at higher wavelengths, hence, VAR content could be analysed at a wavelength of 320 nm.

Consequently, for varenicline analysis, we chose a methanol-buffer solution pH 3.5 (0.5 mmol/l solution of sodium benzoate adjusted to pH 3.5 with 20 mmol/l solution of trifluoroacetic acid, 55:45 *V/V*) pumped with a flow rate of 1.2 ml/min. In the experiment, the column temperature was kept constant at 22.5 ± 1°C. Under these chromatographic conditions, the retention time of VAR was 2.5 min (RSD 0.13%, n = 10) (Figure 2).

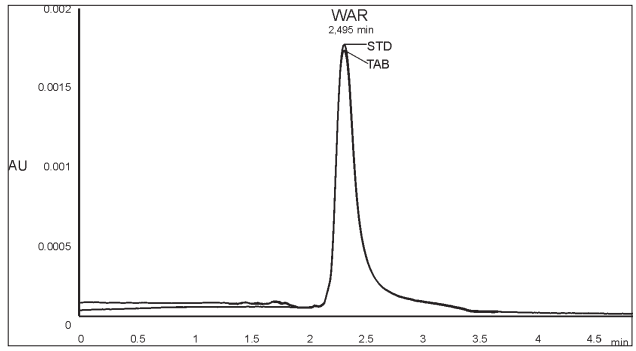

Figure 2. Typical chromatogram of VAR standard solution and VAR solution of tablet extract (VAR concentration of 10 μg/ml)

System suitability. We tested the system's suitability for being an integral part of an analytical procedure. This was done with respect to the capacity factor, injection repeatability, tailing factor, theoretical plate number and the signal-to-noise ratio for the VAR peak using a VAR solution of 10 μg/ml. In such work, the capacity factor was found to be 4.1 - indicating that the VAR peak is well resolved with respect to the void volume. Moreover, the RSD of five consecutive injections was found to be less than 0.2% - indicating good injection repeatability. In addition, the tailing factor was found to be 1.065 - reflecting good peak symmetry. What is more, the theoretical plate number was found to be 3083 for the column used in the study (Chromolith Performance RP18e, 100 × 4.6 mm, macropores 2 μm, mesopores 13 nm) - thus demonstrating satisfactory column efficiency. Finally, 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 HPLC 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 analyzed substance, and the retention times of the degradation products were different from those of VAR. In the case of oxidation, one peak at retention time

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 injected in duplicate, 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 0.2-10 μg/ml. The calibration equation was: AU (detector signal) = 28396936 C (concentration of VAR) + 781 with the determination coefficient of 0.9999. 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), while normality was assessed by applying the Shapiro-Wilks test (TV= 0.9574, p = 0.26). 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.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the signal-to-noise method [17]. 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 10 and 33 ng/ml, (respectively).

Precision was measured in accordance with ICH recommendation [17]. Six subsequent injections of VAR solution at three different concentrations (9, 3, and 0.3 μg/ml) showed acceptable intra-day precision; the relative standard deviations (RSD, n = 6) 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 = 12) of less than 3% indicate acceptable reproducibility of the method (Table 1).

Table 1. Precision of the proposed method

The labelled concentration (μg/ml)	The found concentration (μg/ml)	RSD %
Intra-day precision (n = 6)		
9.0	9.009	0.54
3.0	3.009	0.67
0.3	0.296	2.59
Inter-day precision (n = 12)		
9.0	8.967	0.42
3.0	3.008	0.69
0.3	0.297	3.08

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 (μg/ml)	The found concentration (μg/ml)	Recovery* %
M150%	1.850	1.873	0.54
M100%	1.250	1.247	0.67
M50%	0.600	0.598	2.59

* mean values, 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 ± 1.31% RSD (intra-day analysis) and 99.46 ± 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. Statistical analysis of the results from VAR determination in Champix tablets (1 mg/tablet) by HPLC method

Parameter	Intra-day analysis (n = 6)	Inter-day analysis (n = 12)
Mean amount found (mg)	0.9996	0.9946
Standard deviation	0.0131	0.0124
Variance	1.71E-04	1.54E-04
Relative standard deviation (%)	1.31	1.25
95% Confidence interval	±0.014	±0.079
Relative standard error (%)	-0.03	-0.53

CONCLUSIONS

The developed HPLC method with DAD detection and Chromolith performance column is selective, sensitive, accurate and precise. Statistical analysis for the results demonstrate 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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