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A Novel Stability Indicating RP-HPLC Assay Method for the Determination of Varenicline in Pharmaceutical Formulations

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Abstract: Background: Varenicline is suggested for use in smoking cessation and very few methods are reported for the determination of varenicline. **Materials and Methods:** A novel, simple, accurate, precise and stability indicating RP-HPLC method has been developed and validated for the assay of Varenicline in pharmaceutical formulations. **Results:** Separation was achieved within 10 min with required asymmetry, accuracy and precision thus enabling the utility of the method for routine analysis. Chromatographic separation was achieved on a Inertsil ODS 3 V 5 μm, 150×4.6 mm using a mobile phase consisting of 0.1% tri fluroaceticacid, methanol and Acetonitrile in the ratio of 8:1:1 at a flow rate of 1.0 mL per min. The detection was made at 235 nm. The retention time of Varenicline is 4.9 min. **Conclusion:** The method was validated and demonstrated good linearity, precision, accuracy and specificity in compliance with the regulatory requirements.

Key words: Varenicline, RP-HPLC, stability, formulations, validation

INTRODUCTION

Smoking is widely recognized as a serious health problem, which is increasing in prevalence across the world. Smoking is a high risk factor for coronary heart disease, chronic obstructive pulmonary disease, cancers and various other health related problems (Singh and Budhiraja, 2008). According to the World Health Organization, more than one billion persons worldwide routinely smoke and despite efforts to aid people in their attempts to quit and the provision of education about the dangers of tobacco use, it is estimated that the number of people who smoke will climb to more than 1.6 billion by the year 2025 (Faulkner, 2009).

Smoking cessation is challenging and behavioral interventions have had only modest success. Drug therapy has been increasingly relied upon to assist in smoking cessation. The most common of these has been Nicotine Replacement Therapy (NRT) and anti-depressant therapy specifically the agent bupropion (Rao and Shankar, 2009). Nicotine replacement therapy includes transdermal patch, gum, inhaler, nasal spray and lozenges (Mohanasundaram *et al*., 2008). Psychological therapies are also used for smoking cessation, such as smoking cessation programs. However, efficacy for these therapies is limited and several shortcomings exist, warranting development of a better treatment option. Even when tobacco users are able to cease using these products for periods of time, it is common for them to reinitiate use habits several months later (Obach *et al*., 2006).

Varenicline is suggested for use in smoking cessation. It is an alternative to NRTs and has demonstrated greater efficacy than them in comparable studies (Krishnan, 2008). Varenicline is the first non-nicotine-containing medication developed with the sole purpose of treating nicotine addiction. It was approved as a prescription-only aid to smoking cessation in 2006 by the American Food and Drug Administration under the trade name Chantix and by the European Medicines Evaluation Agency under the trade name Champix (Rao and Shankar, 2009).

Varenicline is a partial agonist of the nicotinic α4β2 acetylcholine receptor, with demonstrated efficacy as a smoking cessation agent (Coe *et al*., 2005). Varenicline is highly selective for the α4β2nicotinic acetylcholine receptor which is responsible for mediating the reinforcing properties of nicotine in the brain. As a partial agonist with higher affinity and less functional effect than nicotine, varenicline may alleviate craving and withdrawal during smoking cessation. With nicotine exposure, the receptor occupancy of varenicline would be expected to

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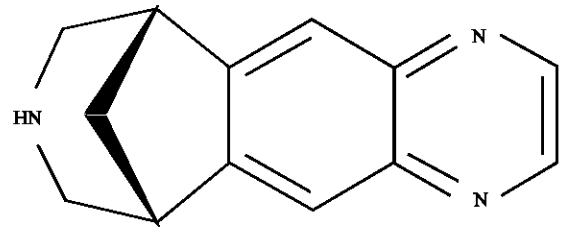

Fig. 1: Chemical structure of varenicline

block the reinforcing effects of smoking. This may lead to an extinction of smoking behavior prior to a quit attempt and prevent a slip from becoming a relapse after a quit attempt.

Varenicline (Fig. 1), is 7,8,9, 10-tetrahydro-6, 10-methano-6H-pyrazino(2,3-h)(3)benzazepine. The empirical formula of Varenicline is $C_{13}H_{13}N_3$ and its molecular weight 211.267. Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light and enables recommendation of storage conditions, retest periods and shelf lives to be established. The two main aspects of drug product that play an important role in shelf life determination are assay of active drug and degradation products generated, during the stability study. The assay of drug product in stability test sample needs to be determined using a stability-indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines (ICH, 1993).

Although the use of varenicline is continuously increasing and very few analytical methods are available to estimate varenicline (Satheesh *et al*., 2010). This study describe a novel, simple, sensitive and validated stability indicating HPLC method utilizing isocratic mobile phase with short retention time for the determination of varenicline in pharmaceutical formulations.

MATERIALS AND METHODS

Chemicals and reagents: Varenicline tartarate working standard was procured from Matrix laboratories, Hyderabad and the tested pharmaceutical formulations (Chantix tablets) were procured from commercial pharmacy. Trifluroacetic acid, Acetonitrile and methanol were of suitable analytical grade.

Apparatus and chromatographic conditions: HPLC analysis was performed on Waters HPLC system equipped with a 2696 separation module and 2996 Photo Diode Array Detector. Separations were carried on Inertsil ODS 3V 5 μm, 150×4.6 mm using isocratic elution. The flow rate was 1.0 mL min^{-1}. UV detection was performed at 235 nm. Injection volume was 100 μL. Peak identity was confirmed by retention time comparison and the HPLC was operated at room temperature.

Preparation of mobile phase: The mobile phase is composed of a mixture of 0.1% of trifluroacetic acid, methanol and acetonitrile in the ratio of 8:1:1 (v/v) filtered through a 0.45 μm nylon filter (Millipore, USA) and degassed by sonication prior to use.

Preparation of standard solution: The standard stock solution of varenacline (0.1 mg mL^{-1}) was prepared in methanol since drug is soluble in this solvent. The working standard solution (5 μg mL^{-1}) was prepared by diluting the stock solution in mobile phase solution.

Preparation of sample solution: Twenty tablets were weighed to get the average weight and grounded. An amount of powder equivalent to 1 mg of Varenicline was transferred to a 100 mL volumetric flask and added 70 mL of diluent and sonicated for 30 min with intermediate shaking. The volume was made up with diluents to obtain a solution containing 10 μg mL^{-1} varenicline. An aliquot was then removed and centrifuged at 5000 rpm for 10 min. The solution was filtered using 0.45 μm membrane filter paper.

RESULTS

Method development: Drug quality control, stability, metabolism, pharmacokinetics and toxicity studies all necessitate the determination of drugs in pharmaceutical formulations and biological samples. Correspondingly, efficient and validated analytical methods are very critical requirements for all these investigations. Chromatographic parameters were preliminary optimized to develop a stability indicating assay method for Varenicline tablets with short analyses time (<10 min).

Varenicline is highly soluble in water, chosen the reverse phase column Inertsil ODS-3V column to obtain good resolution. In order to identify a suitable organic modifier, various compositions of acetonitrile and methanol were tested. Finally 1:1 ratio of Methanol and Acetonitrile was chosen as the organic modifier. In order to get shorter analyses time, various composition of buffer and organic modifier was tried. In higher organic modifier composition (more than 20%), peak purity of Varenicline was found to be not meeting the acceptance

criteria and finally chosen the 20% of organic modifier in the mobile phase. Different buffers were tried to get good peak shape and less retention time, finally the 0.1% trifluro acetic acid was chosen which was giving sharp peak compare to other buffers.

Different levels of injection volumes were tried to get good response due to the lower strength of the product and finally selected 100 µL as the injection volume. Sample was scanned from 200 to 400 nm to select the operating wavelength and selected 235 nm as λmax (Fig. 2).

Method validation: The above method was validated according to ICH and USP guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method.

System suitability: In order to determine the adequate resolution and reproducibility of the proposed methodology, suitability parameters including retention time, resolution, Tailing factor, %RSD of retention time and peak areas were investigated. The results are summarized in Table 1. The Tailing factor and % RSD of retention time were found to be 1.3 and 0.2, respectively.

Specificity: The specificity of an analytical method may be defined as the ability to unequivocally determine the analyte in the presence of additional components such as impurities, degradation products and matrix components. Specificity was evaluated by preparing the analytical placebo sample, standard solution and sample of commercial pharmaceutical formulation.

A solution of analytical placebo (containing all the tablet excipients except Varenicline) was prepared according to the sample preparation procedure and injected. To identify the interference by these excipients, standard solution and the commercial pharmaceutical preparations were also analyzed. The placebo chromatograms did not show any other peaks which confirmed the specificity of the method (Fig. 3, 5). Peak purity of varenicline was evaluated by peak purity profiles (Fig. 6, 7). The peak purity profiles confirm the purity of the peaks since the purity angle is more than purity threshold for both test and standard preparations. The results are summarized in Table 2. The purity angle and purity threshold of standard were found to be 0.042 and 0.233, respectively. The The purity angle and purity threshold of sample were found to be 0.036 and 0.318, respectively.

Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which

Table 1: System suitability

Parameter	Result	Acceptance criteria
Tailing Factor	1.3	NMT 2.0
%RSD of Peak Area	0.2	NMT 2.0%

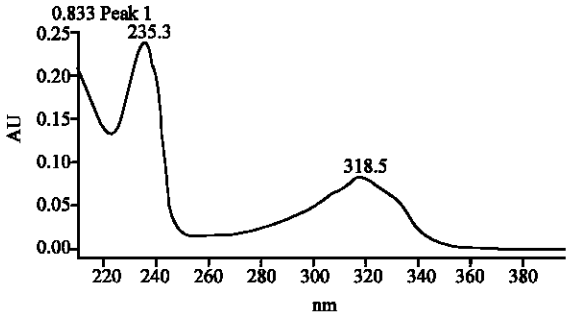

Fig. 2: Spectrum of varenicline

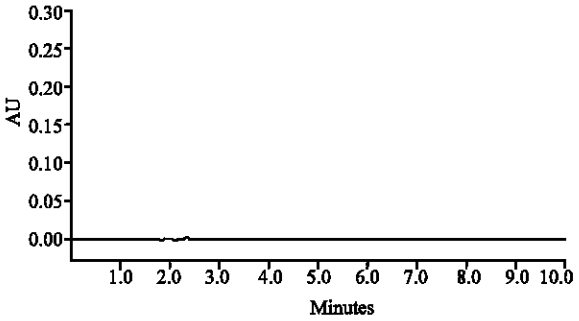

Fig. 3: Chromatogram of placebo

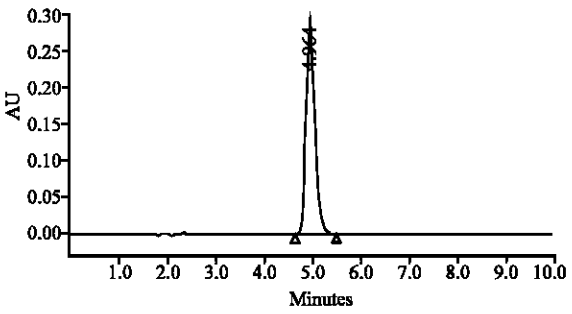

Fig. 4: Chromatogram of standard

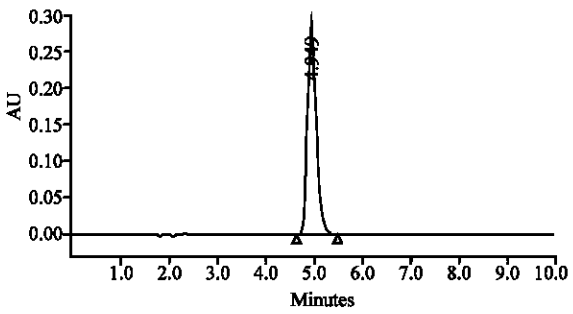

Fig. 5: Chromatogram of sample

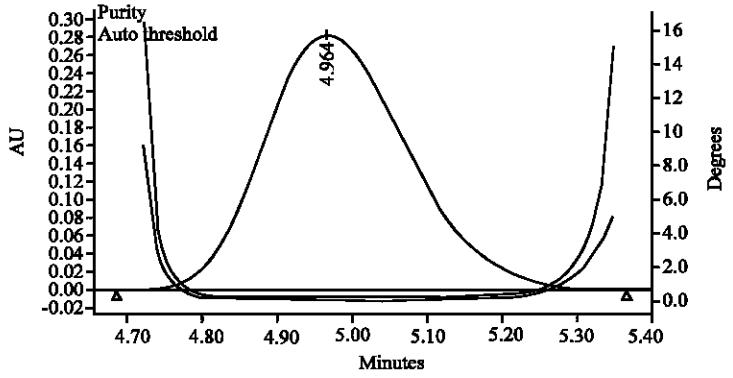

Fig. 6: Peak purity plot of standard

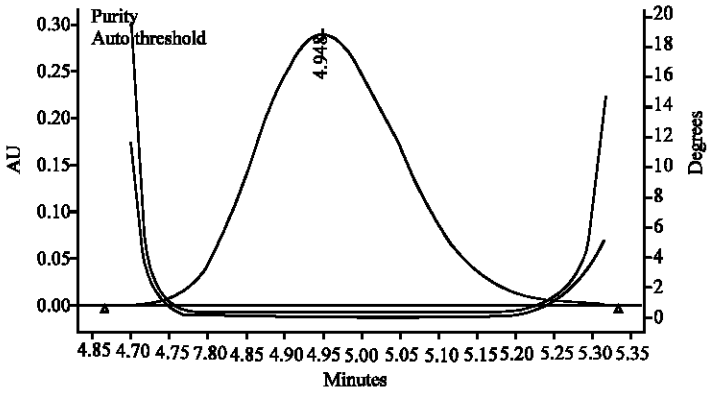

Fig. 7: Peak purity plot of sample peak

are directly proportional to the concentration (amount) of analyte in the sample. Linearity of detector response for varenicline was established by analyzing serial dilutions of a stock solution of the working standard. Ten concentrations ranging from 2.5 to 7.5 μg $mL^{-1}$of the test concentration were prepared and analyzed. The final concentration of each solution in μg per mL was plotted against peak area response. Correlation coefficient was found to be greater than 0.999. The linearity details are presented in Table 3.

Accuracy and precision: Accuracy is the the degree of closeness of the determined value to the nominal or known true value under prescribed conditions and precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Six replicate samples were prepared and analyzed as per the sample preparation procedure. Assay of each replicate, the average of 6 replicates, its standard deviation, %RSD and the 95% confidence interval were calculated. The results are shown in Table 4.

Table 2: Standard and sample peak purity

Injection	Purity angle	Purity threshold	Peak purity
Standard	0.042	0.233	Passes
Sample	0.036	0.318	Passes

Table 3: Linearity

Concentration (μg mL^{-1})	Area
2.5	1920456
3	2303248
3.5	2681359
4	3078934
5	3845426
5.5	4228935
6	4612465
6.5	4998363
7	5373245
7.5	5765632
Slope	769000
R	0.9999

Recovery: Recovery study was performed at 50, 75, 100, 125 and 150% of the target concentration by spiking placebo blend with the drug substance. Six replicates each

Table 4: Accuracy and precision

	Assay	
Sample No.	0.5 mg	1 mg
1	101.3	100.8
2	100.6	101.7
3	100.6	101.2
4	100.7	100.9
5	100.9	100.9
6	101.0	100.6
Mean ($\bar{X}$)	100.9	101.0
S.D.	0.2739	0.3869
%RSD	0.30	0.40
Lower 95% CI	100.7	100.7
Upper 95% CI	101.1	101.3

were spiked at 50 and 150% levels and 3 replicates each at 75, 100 and 125% levels. Spiked samples were extracted and analyzed. The amount spiked, amount recovered, percent recovery and its mean were calculated. The mean recovery across all the levels ranged from 99.6 to 100.7%.

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The variations like flow rate of mobile phase, column temperature; ratio of organic content in the mobile phase etc. does not have any significant effect on the method performance.

DISCUSSION

A novel, simple, rapid, cost effective and accurate RP-HPLC method was developed for the Varenicline in pharmaceutical formulations by isocratic mode elution. The retention time of varenicline was found to be 4.9 min with the total run time of 10 min. The HPLC method was validated and demonstrated good linearity, precision, accuracy and specificity. The results of the validation parameters were found to be in compliance with regulatory specifications. Thus, the developed HPLC method can be utilized for quality control testing of Varenicline in pharmaceutical formulations and in drug substances.

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REFERENCES

Coe, J.W., P.R. Brooks, M.G. Vetelino, M.C. Wirtz and E.P. Arnold *et al*., 2005. Varenicline: An α4β2 nicotinic receptor partial agonist for smoking cessation. J. Med. Chem., 48: 3474-3477.

Faulkner, M.A., 2009. Smoking cessation: An economic analysis and review of varenicline. Clin. Econ. Outcom. Res., 1: 25-34.

ICH, 1993. Stability testing of new drug substances and products. International Conference on Harmonization-Guidance for Industry, Geneva.

Krishnan, B., 2008. Smoking cessation: Initial insights into a new drug. Kathmandu Univ. Med. J., 2: 20-23.

Mohanasundaram, U.M., R. Chitkara and G. Krishna, 2008. Smoking cessation therapy with varenicline. Int. J. Chron. Obstruct. Pulmon. Dis., 3: 239-251.

Obach, R.S., A.E. Reed-Hagen, S.S. Krueger, B.J. Obach and T.N. O'Connell *et al*., 2006. Metabolism and disposition of varenicline, a selective α4β2 acetylcholine receptor partial agonist, *in vivo* and in vitro. Drug. Metbol. Dispost., 34: 121-130.

Rao, J. and P.K. Shankar, 2009. Varenicline: For smoking cessation. Kathmandu Univ. Med. J., 7: 162-164.

Satheesh, B., S. Kumarpulluru, V. Raghavan and D. Saravanan, 2010. UPLC separation and quantification of related substances of varenicline tartrate tablet. Acta Chromatogr., 22: 207-218.

Singh, J. and S. Budhiraja, 2008. Partial nicotinic acetylcholine (α4β2) agonists as promising new medications for smoking cessation. Indian J. Pharmacol., 40: 191-196.