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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF VARENICLINE TARTRATE IN BULK DRUG AND TABLET DOSAGE FORM

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ABSTRACT

A reverse phase high performance liquid chromatographic method has been developed and validated for the estimation of Varenicline tartrate in bulk and tablet using UV detector. Gradient chromatography was performed on a C-18 column, with a mobile phase composed by Methanol: Potassium dihydrogen orthophosphate buffer pH 3 (50:50, v/v), at flow rate of 0.6 ml / min using UV detection at 237 nm. The retention time for Varenicline tartrate was found to be 2.966 min. Linearity of the method was found to be 10 to 50 µg/ml, with the regression coefficient of 0.9999. This method was validated according to ICH guidelines. The intra-day and inter day percentage relative standard deviation (RSD) was found 0.327 and 0.147 respectively. The proposed method was successfully applied for the quantitative determination of Varenicline tartrate in tablet formulations.

Keywords: Varenicline tartrate, RP-HPLC, Gradient, Tablet formulation.

INTRODUCTION

Varenicline tartrate [1, 2] is a novel anti-smoking for oral administration. It is designated 7,8,9,10-tetrahydro-6,10-methano-6H-pyrazino[2,3-h][3]benzazepine(2R,3R)-2,3-dihydroxybutane-dioate. Varenicline tartrate is the first approved nicotinic acetylcholine receptor partial agonist. Specifically, Varenicline tartrate is a partial agonist of the $\alpha_4\beta_2$ subtype of the nicotinic acetylcholine receptor. In addition it acts on $\alpha_3\beta_4$ and weakly on $\alpha_3\beta_2$ and α_6-containing receptors. A full agonism was displayed on α_7-receptors. It is a prescription medication used to treat smoking addiction. Structure formula of Varenicline tartrate given below

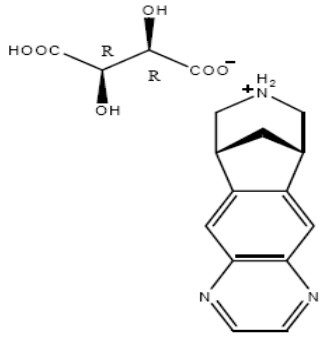

Fig. 1: Structure formula of Varenicline tartrate

Analytical methods are required to characterize drug substances and drug products composition during all phases of pharmaceutical development. Varenicline tartrate is not official in any pharmacopoeia. Extensive literature survey reveals that only one UPLC method [3] was reported for determination of Varenicline tartrate, but no other methods were reported for determination of Varenicline tartrate, like UV-Visible spectrophotometric, HPLC, HPTLC, etc.

The present method describes a new quantitative reverse phase high performance liquid chromatographic method coupled with UV detector, as an alternative technique for quality control of Varenicline tartrate products. The developed revers phase high performance liquid chromatographic technique was simple, precise, specific and accurate and the statistical analysis proved that, method is reproducible and selective for the analysis of Varenicline tartrate in bulk drug and tablet formulation.

MATERIALS AND METHODS

Reagents and chemicals

An analytically pure sample of Varenicline tartrate was procured as gift sample from Watson Pharma, (Mumbai, India). High performance liquid chromatographic grade methanol was procured from E. Merck (Ahmedabad). Liquid chromatographic grade water was obtained by double distillation and purification through Milli- Q water purification system. Potassium dihydrogen orthophosphate (AR grade, purity 99.5 %) was procured from Qualigens. Tablet formulations (champix) were procured from a Watson pharma, Mumbai with labeled amount 1 mg per tablet.

Chromatographic equipments

The liquid chromatographic system consisted of following components: High performance liquid chromatography equipped Waters-2695 separation module with auto sampler and Waters-2487 dual λ-absorbance UV detector. The data processing was performed using EM-power software. Chromatographic analysis was performed on a Kromasil C-18 column with 250 x 4.6 mm i.d. and 5 µm particle size.

Chromatographic conditions

A RP C-18 column equilibrated with mobile phase Methanol: Potassium dihydrogen orthophosphate buffer pH 3 (50:50, v/v) was used. Mobile phase flow rate was maintained at 0.6 ml / min. Detection wavelength 237 nm was selected by scanning standard drug over a wide range of wavelength 200 nm to 400 nm in spectrophotometer. The 20 µl sample was injected by an auto sampler, and the total run time was 5 min.

Preparation of standard solutions

Accurately 10 mg of Varenicline tartrate was Weighed and transferred to a 100 ml volumetric flask. To this mobile phase was added and sonicated for 15 minutes. This yielded a working standard solution with concentration 100 µg/ml of Varenicline tartrate. This working standard solution was diluted to give solutions of concentration 10-50 µg/ml.

Sample preparation

Twenty tablets of Varenicline tartrate were powdered and powder equivalent to 1 mg of Varenicline tartrate was taken in 100 ml volumetric flask. Add about 20 ml of diluent and sonicated to dissolve completely. The final volume was made up to 50 ml to get a

stock solution of 20 µg/ml. This solution was filtered through a 0.45µm membrane filter.

RESULTS AND DISCUSSION

The proposed method is simple and less time consuming for sample preparation and method was statistically proved for their accuracy and precision. Chromatogram of standards and formulation are given in **fig. 2** and **3** respectively.

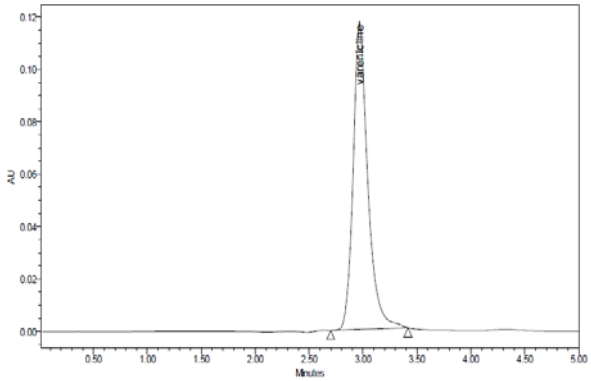

Fig. 2: Chromatogram of Varenicline tartrate standard (20 µg/ml)

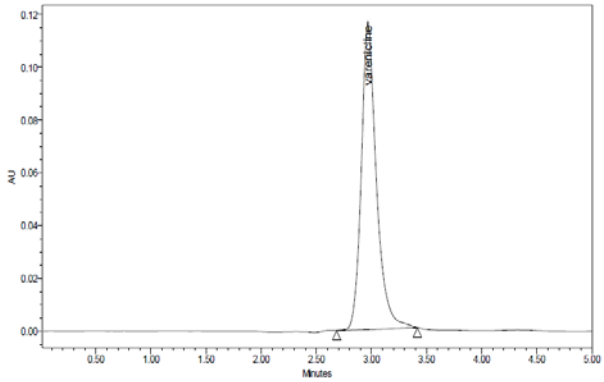

Fig. 3: Chromatogram of Varenicline tartrate formulation (20 µg/ml)

Method validation

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures [4, 5]

System suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (*RT*), number of theoretical plates (*N*), tailing factor (*T*), and peak asymmetry (*As*) were evaluated for five replicate injections of the drug at a concentration of 20 µg/ml. The results given in **Table 1** were within acceptable limits.

Table 1: Results from system suitability studies

Property	Values*	Required limits
Retention time (*RT*)	2.966 ± 0.00125	RSD ≤ 1%
Theoretical plates (*N*)	2518.152 ± 98.77	*N* > 2000
Tailing factor (*T*)	1.26 ± 0.132	*T* ≤ 2
Asymmetric(*As*)	1.11±0.002	*As* ≤ 1.5

* Mean ± S.D. from six determinations

Linearity and range

Appropriate aliquots of standard Varenicline tartrate stock solutions (100 µg/ml) were taken in different 10 mL volumetric flask and resultant solution was diluted up to the mark with diluent to obtain final concentration of 10-50 µg/ml. The solutions were prepared in triplicate. Calibration curve were constructed by plotting the concentration of Varenicline tartrate versus corresponding mean peak area. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range. The corresponding linear regression equations, with correlation coefficient (r^2) = 0.9999, was y = 56098x + 12374. The results given in Table 2 and Fig. 4.

Table 2: Calibration data of Varenicline tartrate by RP-HPLC method

Sr. No.	Concentration (µg/ml)	Retention time (min)	Peak area (mv)
1	10	2.966	567859
2	20	2.966	1134641
3	30	2.967	1705850
4	40	2.965	2256311
5	50	2.966	2811931

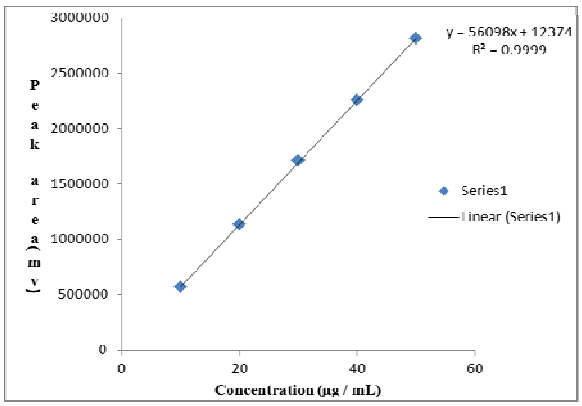

Fig. 4: Calibration curve of Varenicline tartrate by RP-HPLC method

Sensitivity

The sensitivity of measurement of Varenicline tartrate by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). The limit of quantitation (LOQ) was 0.009 µg/ml and the limit of detection (LOD) was 0.003 µg/ml.

Precision

The intra-day precision was determined by analyzing standard solution of concentration 20 µg/ml for 6 times on the same day while inter-day precision was determined by analyzing corresponding standards daily for 6 day over a period of one week. The values of percentage relative standard deviation (% RSD) for intra-and inter-day variation are given in **Table 3**.

Table 3: Results from precision studies

Sr. No.	Concentration (µg/ml)	Intraday precision (Area)	Interday precision (Area)
1	20	1113501	1137148
2	20	1109222	1136191
3	20	1114446	1135971
4	20	1118765	1134981
5	20	1119018	1136477
6	20	1115467	1132452
Mean		1115070	1132452
Std. Dev.		3645.48	1668.61
% RSD		0.327	0.147

Accuracy

Accuracy of the method was checked by recovery study using standard addition method; known amount of standard Varenicline tartrate was added into pre analyzed sample and subjected it to the proposed high performance liquid chromatographic method. These studies were carried out at three levels i.e., multiple level recovery studies. The recovery studies were carried out and the % recovery and standard deviation of the % recovery were calculated and presented in **Table 4**.

Table 4: Results from recovery studies

Brand	Label claim (mg)	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	Recovery ± SD* (%)	% RSD
	1	20	10	10.116	101.16 ± 0.20	0.203
Champix	1	20	20	20.23	101.15 ± 0.41	0.412
	1	20	30	30.55	101.83 ± 0.15	0.149

*Average of six determinations

Ruggedness and robustness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst. The results of ruggedness testing are reported in the **Table 5.**

Robustness is a measure of capacity of a method to remain unaffected by small but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at two different levels and chromatographic response was evaluated. The Organic composition in the Mobile phase was varied from 45% to 55%, and the variation in mobile phase flow rate by 0.6 ml / min (0.5 and 0.8 ml / min) had no significant effect on the retention time and chromatographic response of the 20 µg/ml solution, indicating that the method was robust. The results are shown in **Table 6.**

Table 5: Results from ruggedness studies

Sample	Label claim (mg)	Analyst I		Analyst II	
		Amount found (mg)	Recovery ± SD* (%)	Amount found (mg)	Recovery ± SD* (%)
Champix	1	0.993	99.3 ± 0.32	1.01	101 ± 0.10

*Average of six determinations

Table 6: Results from the robustness studies

Condition	Modification	Mean area ± SD*	RSD (%)	Mean RT ± SD* (min)
Change in Organic	5% Less	1165778 ± 5414.551	0.657	2.962 ± 0.034
Composition of Mobile	Actual	1105780 ± 3504.117	0.424	2.968 ± 0.011
Phase	5% More	1112948 ± 4633.691	0.560	3.159 ± 0.045
Mobile phase flow rate	0.5	1373043 ± 4207.045	0.510	3.551 ± 0.004
(ml /min)	0.6	1103278 ± 3804.307	0.462	2.967 ± 0.010
	0.8	972356 ± 524.929	0.668	2.560 ± 0.067

*Average of six determinations

CONCLUSION

The devolved RP-HPLC method was simple, sensitive, precise and accurate, hence can be used in routine for the determination of Varenicline tartrate in bulk as well as pharmaceutical preparations.

ACKNOWLEDGMENT

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