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Development and validation of related substances method for Varenicline and its impurities

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ABSTRACT

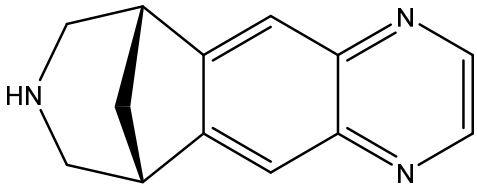
A simple and accurate reverse phase HPLC method has been developed and validated for quantification of Varenicline and its related impurities in bulk drug and pharmaceutical dosage form. Separation was achieved on c18 column by mobile phase consisted phosphate buffer with pH 3.0 and acetonitrile and flow rate 1.0ml/min. The detection wavelength was set at 235nm. The method was linear at concentration range from LOQ to 150% of specification level. The limit of quantification and limit of detection values less than 20% of specification level. Method precision and ruggedness the relative standard deviations did not exceed 2%. The accuracy of the method proved, the mean recovery between 85 to 115%. The results demonstrated that the method would have a great value when applied in quality control and stability studies for Verenicline.

Keywords: Varenicline. Development. Validation. HPLC

INTRODUCTION

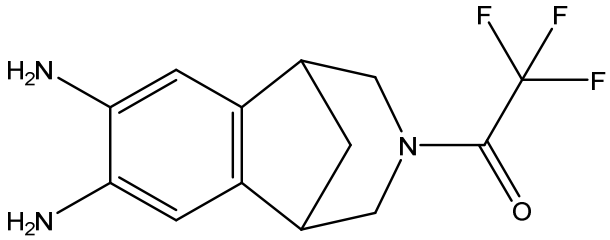
Varenicline is a prescription medication used to treat nicotine addiction. Varenicline is a nicotinic receptor partial agonist. In this respect it is similar to cytosine and different from the nicotinic antagonist, bupropion and nicotine replacement therapies like nicotine patches and nicotine gum. As a partial agonist, it both reduces cravings for and decreases the pleasurable effects of cigarettes and other tobacco products. Through these mechanisms, it can assist some patients to quit smoking. Varenicline tartrate has a molecular weight of 361.35 daltons, and molecular formula of $C_{13}H_{13}N_3.C_4H_6O_6$.

Literature serve revels that related compounds by UPLC (1,2), assay by HPLC (3,4,5) methods have been reported for Varenicline. The present work was designed to develop a sensitive, precise, linear and rugged RP-HPLC method for the quantification of Varenicline and its related impurities in bulk drug and formulation. The method is validated as per the guidelines of the international conference on harmonization (ICH).



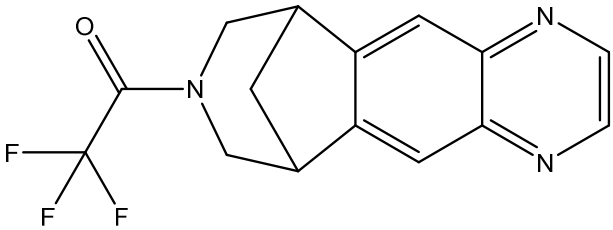
(6*R*,10*S*)-7,8,9,10-tetrahydro-6*H*-6,10-methanoazepino[4,5-*g*]quinoxaline

Fig.1 Varenicline



1-(4,5-Diamino-10-aza-tricyclo[6.3.1.02,7]dodeca-2(7),3,5-triene-10-- yl)-2,2,2-trifluoro-ethanone

Fig.2 IMP-I



ethanone, 2,2,2-trifluoro-1-(6,7,9,10-tetrahydro-6,10-methano-8H-pyrazino[2,3-h][3] benzazepin-8-yl)

Fig.3 IMP-II

MATERIALS AND METHODS

Chemicals and Reagents

The HPLC grade Acetonitrile, Methanol and analytical grade potassium di hydrogen phosphate and phosphoric acid were purchased from Merck, Mumbai. High purity water was prepared by using a Millipore Milli Q plus water purification system. High purity of all impurities namely IMP-I, IMP-II and Varenicline as a gift.

Chromatographic conditions

Alliance e2695 separation module (Waters Corporation, Milford, MA) equipped with 2998 PDA detector with empower 2 software used for analysis. The method was developed using Atlantis dC18 (250x4.6) mm, a 5µm column with a mobile phase consist a gradient mixture of A and B. 2.72 g of potassium di hydrogen phosphate in 1000ml of water pH adjusted 3.0 with diluted phosphoric acid was used as solvent A and methanol was used as solvent B. The gradient program was set as (T/%B) 0/20,7/20,20/50,25/5025.5/20 and 30/20.The mobile phase flow rate was set as 1.0ml/min and the temperature was maintained as 45ºC. The injection volume was 30µl and the wavelength was monitored at 235nm. Solution A: Acetonitrile ratio (80:20) was used as diluent.

Preparation of standard and sample solutions

A working solution containing about 0.2 µg/ml of impurity-I, 0.75µg/ml of impurity-II and 0.5µg/ml of Varenicline was prepared for related substances determination. A sample solution of Varenicline (0.5mg/ml) is prepared by dissolving the appropriate amount of Varenicline solid in the diluent. The drug product equivalent to 50mg of sample is transferred to 100ml flask dissolved and diluent volume with diluent (0.50mg/ml). This solution then filtered through a 0.45µ nylon membrane filter.

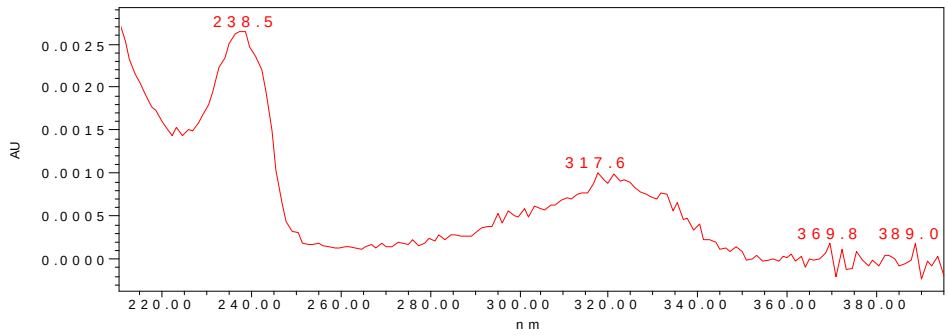


Fig-4 wavelength spectrum of Varenecline

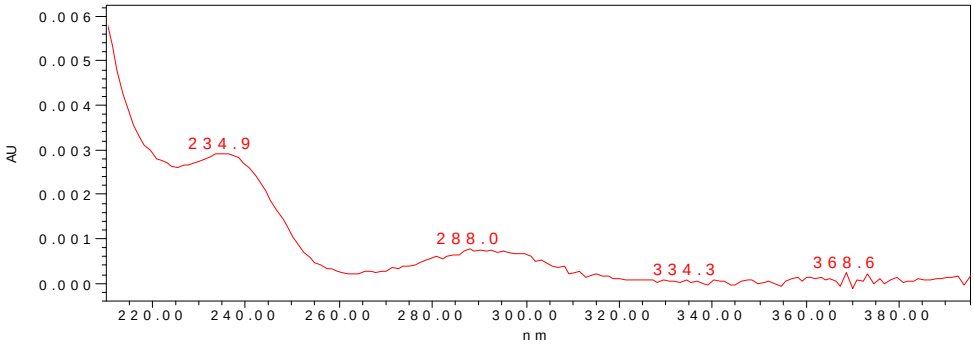


Fig-5 wavelength spectrum of Impurity-I

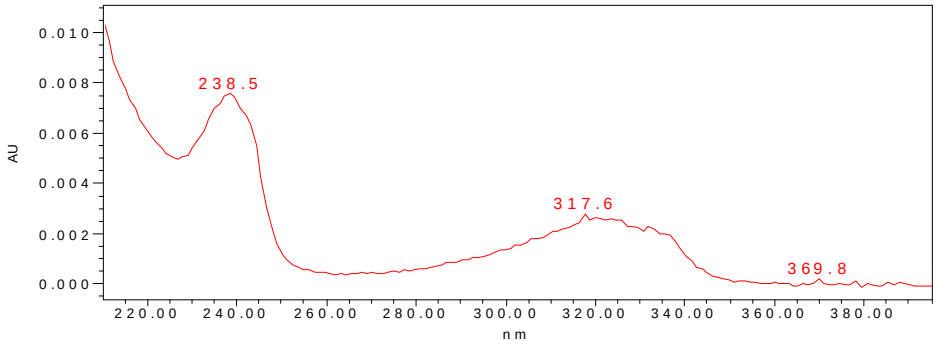


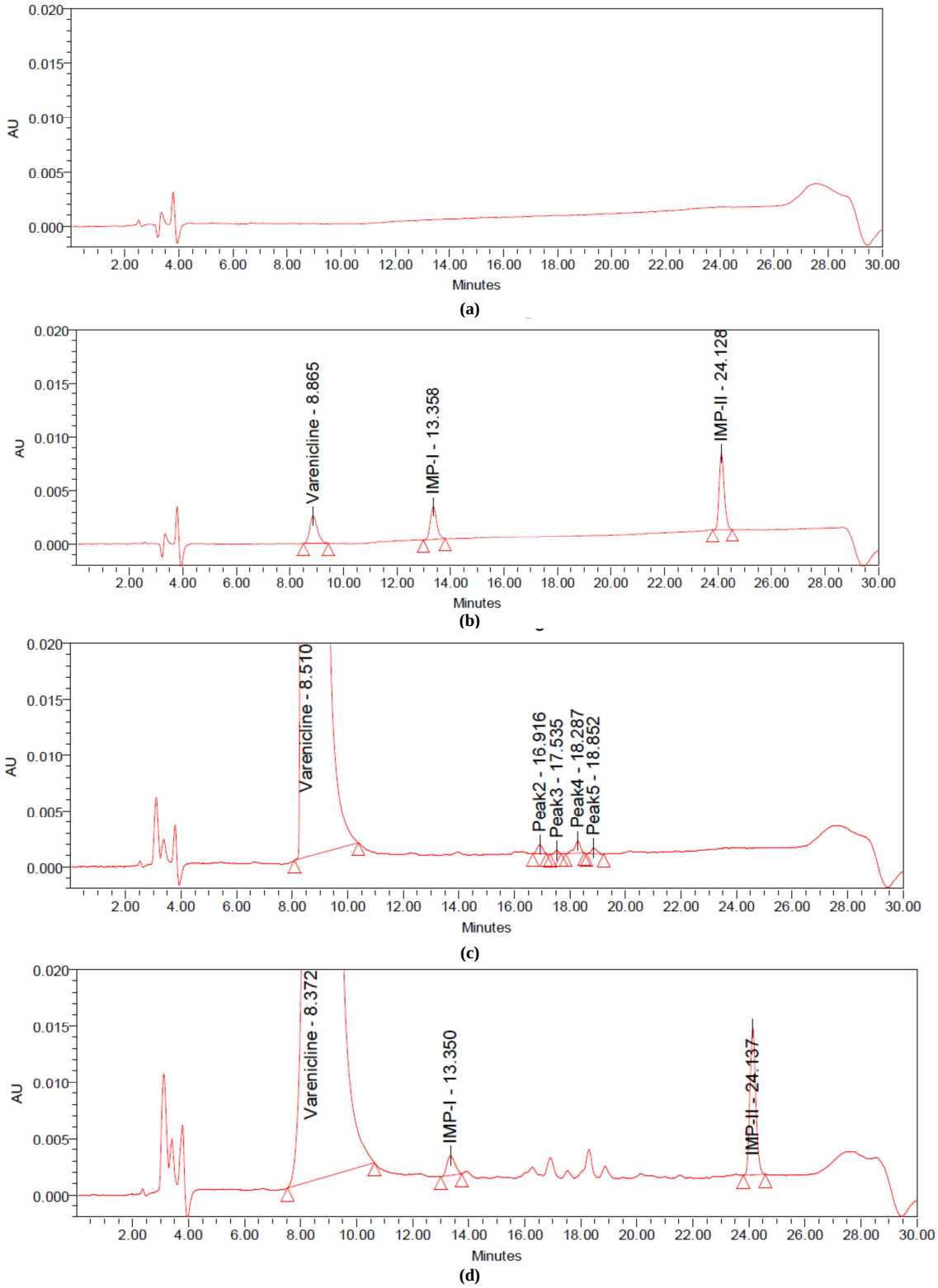
Fig-6 wavelength spectrum of Impurity-II

Method development and optimization

The main difficulty in this study was to get symmetrical peaks for all the analytes and better separation between impurities and Varenicline in a single method without any interference. Led to peak tailing the presence of the amine group functionality in Varenicline and impurities. Different stationary phases like C8,phenyl, and cyno were tried by using gradient elution method with phosphate buffer with different pH, as part of method development. Found that peak shapes were not symmetrical, different ionic strengths were employed with phosphate, acetate buffers reduce the peak tailings, whereas ionic strength no impact on peak tailing.

The different chromatographic conditions like column temperature (25-50ºC) and column particle size (3.5µm and 5.µm) incorporation to improve peak shapes and resolution. Symmetrical peak shapes and resolution was achieved by reverse phase Atlantis dC18 (250x4.6) mm, a 5µm column operated at 45ºC with gradient elution at 1.0ml/min using mobile phase A as 2.72 g of potassium di hydrogen phosphate in 1000ml of water pH adjusted 3.0 with diluted phosphoric acid. Mobile phase B consisting methanol, the detection wavelength was at 235nm (fig.4-6) and injection volume was 30µl. The gradient program was set as (T/%B) 0/20, 7/20, 20/50, 25/50 25.5/20 and 30/20. All

the impurities were well separated with Varenicline, resolution greater than 3.0 and symmetrical peaks were observed(fig.7(a-d)).



(a)

(b)

(c)

(d)

Fig-7 typical chromatograms of (a) Blank, (b) standard (c) sample (d) spiked

Method validation
The propose method was validated as per the international conference on Harmonization (ICH) guidelines (6-7).

Precision
The Method precision is evaluated by carrying out six independent preparations of a test sample of Varenicline spiked to 0.04% imp-I, and 0.15% imp-II. The %RSD of the areas of impurities calculated. The intermediate precision of the method is evaluated using a different analyst, different column and different instrument. Impurity-I and Impurity-II %RSD is 1.3 and 0.6 indicating that method more precise, similarly 1.0 and 1.1 for intermediate precision results indicating that the method is rugged. Results are tabulated in table-1.

Limit of detection and Limit of Quantification
The LOD & LOQ were calculated for each analyte as a signal-to-noise ratio (S/N) of approximately3: 1& 10:1. It determined by the series of dilution of standard solution. The LOQ values are 0.02, 0.01 and 0.011of Varnecline, Imp-I and Imp-II, similarly 0.006, 0.003 and 0.003 are LOD values. The precision study is carried out at the LOQ level by injecting six individual preparations of varnecline, imp-I, and imp-II, %RSD are 1.1, 1.6 and 2.1 showing method is precise at LOQ level. Results are shown in tabulated in table-1.

Linearity
Linearity test is at six concentration levels from LOQ to 150% of Varenicline and impurity concentration. The linear calibration plot for the method is obtained over the tested calibration range and obtained correlation coefficient is greater than 0.999. The results revealed an excellent correlation between the peak area and analyte concentration. Indicating that method is Linear, results are tabulated in table-1.

Accuracy
Similarly the accuracy is calculated for the recoveries of impurities by the method of standard additions. A known amount of impurities were added to a pre-quantified sample and amount of impurities were estimated. Accuracy of the method is evaluated in triplicated at four concentration levels LOQ levels, 50, 100 and 150%. Recovery range from 96.6 to 110.1 showing that method is accurate at LOQ to 150% level .Results are tabulated in table-1.

Table-I validation results

Validation parameter	Varenicline	Imp-I	Imp-II
System Precision			
%RSD of peak area	1.05	1.62	0.7
Resolution		9.03	26.42
Tailing factor	1.24	1.15	1.21
Column efficiency	4221	14709	74907
Linearity			
Slope	103037	244388	124083
Intercept	0.2	1.0	0.8
r2	0.999	0.9998	0.9995
Quantitation limit (%)	0.02	0.01	0.011
Detection limit (%)	0.006	0.003	0.003
Precision at QL	1.1	1.6	2.1
Accuracy mean%recovery at			
QL	NA	97.5	110.1
50%	NA	96.6	97.2
100%	NA	98.9	98.5
150%	NA	98.7	100.1
Method precision	NA	1.3	0.6
Intermediate precision			
%RSD	NA	1.0	1.1

Robustness
On the robustness of the method, experimental conditions were deliberately altered and the resolution between Varenicline and its impurities and tailing factor for Varenicline and its impurities were recorded. The flow rate of the mobile phase was 1.0ml/min, to study the effect of flow rate on the resolution. Flow was changed by 0.2units from 0.8 to 1.2ml/min. The effect of the column temperature on resolution studied at 40ºC and 50ºC and found that the method is robust.

Solution stability

Solution stability of Varenicline, and its impurities in the related substance by leaving the test solution and the solution spiking with the impurities at the spec level with respect to analyte concentration in a volumetric flask at room temperature for 24hrs. The solution was stable up to 24hrs.

CONCLUSION

A simple, new, sensitive, accurate and robust HPLC method has been developed and validated for the determination of Varenicline and its impurities in bulk drug and dosage form. The method is sensitive intems of low level detection and low level quantification values. Hence the method can be used for routine analysis of quality control.

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