STABILITY INDICATING METHOD OF RELATED IMPURITIES IN VENLAFAXINE HYDROCHLORIDE SUSTAINED RELEASE TABLETS

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ABSTRACT

Recently several methods have been developed for the determination of drugs and their impurities products by Liquid chromatography (HPLC) and Gas chromatography (GC). The HPLC method is described for the determination of venlafaxine impurities in Venlafaxine hydrochloride sustained tablets dosage form. The analyte is contained in a placebo matrix with sustained release form and with a high amount of non-soluble excipients, some of which can interfere with the analysis. This makes their separation and analysis of the impurities.Moreover,due to sustained release form there is a great difficulty to extract the impurities from sample matrix without active degradation.Venlafaxine impurities was analysed using a reversed-phase Inertsil ODS-3V (C18) (250 mm x 4.6 mm , 5µm) column with a mixture of phosphate buffer pH 2.8(Dissolve 4.08 gram of potassium dihydrogen phosphate in 1000 mL of water, add 1 mL of N,N-dimethyl octylamine and adjust pH to 2.8 with ortho phosphoric acid) and Acetonitrile (80:20 v/v). Quantitation was achieved with UV detection at 225nm,pump flow rate was 0.8ml/minutes,sample injection volume 10µL,30°C Column temperature maintained. Calibration curves are linear at concentration ranges of 0.1-5µg/ml(r=0.9999).The excipients powder interference could be eliminated by selection of diluents for sample preparation. This method is validated in terms of specificity, linearity, precision, accuracy and robustness. The procedure prescribed here are Isocratic, simple, selective and can be used for routine quality control and stability indicating tests involving the analysed compound formulated in complex matrix.

KEYWORDS: *Venlafaxine hydrochloride, related impurities, Reversed-phase HPLC; Dosage forms*

INTRODUCTION

Venlafaxine hydrochloride is a structurally novel antidepressant; the mechanism of the antidepressant action of Venlafaxine in humans is believed to be associated with its potentiation of neurotransmitter activity in the CNS. Preclinical studies have shown that Venlafaxine and its active metabolite, O-desmethylvenlafaxine (ODV), are potent inhibitors of neuronal serotonin and norepinephrine reuptake and weak inhibitors of dopamine reuptake[1].

The related substances method for determination of related impurities in Venlafaxine hydrochloride sustained release tablets is developed in house and is validated as per ICH Q2 (R1) and United States pharmacopoeia.

The Stability indicating method developed inhouse for the determination of related impurities in Venlafaxine hydrochloride sustained release tablets is based on the reversed phase liquid chromatographic technique in which the stationary phase is non polar and the mobile phase is polar[6].

The Liquid chromatography (HPLC) technique is used to qualify and to quantify most of the pharmaceutical ingredients. This technique is used to assay the active component and to quantify the related compounds present in the dosage form. The basic principle of this technique is purely based on the interaction of the analyte components with the stationary phase. The analyte will be retained on the stationary phase if it has more "affinity" with the stationary phase.

The analyte will be eluted from the stationary phase (Column) with the constant flow of the eluent (Mobile phase.) After elution, the analyte will be detected with a suitable "Detector" [6].

The quantification of the analyte will be done by comparing the response of the analyte with known concentration standard response. The related substances (Impurities) shall be quantitated in the similar manner. The stability indicating ability of the method will be tested by checking the interferences of the impurities with the analyte and the interference of the impurities with each other, which is also confirmed by the forced degradation study. Each impurity will be quantitated against known concentration standard [6].

The analytical method consists of buffered mobile phase (Buffered to maintain constant pH) and Octadecyl silane silicagel column (Symmetry C18 250 mm x 4.6 mm, 5µm is suitable) as stationary phase. The column temperature is maintained at 30°C and a detection wave length of 225nm. 10µl of sample will be injected on to the column. The components will get separated based on relative "affinity" towards the stationary phase.

The purpose of this report is to outline the Stability indicating method for the determination of related impurities in Venlafaxine hydrochloride sustained release tablets USP/ICH Guidelines.

MATERIALS AND METHOD

Instrumentation
Agilent 1100 series integrated high performance liquid chromatographic system was used for this experiment. Agilent 1100 series system equipped with Agilent1100 series quaternary pump, Agilent 1100 series auto sampler, Agilent 1100 series variable wavelength detector, Agilent 1100 series Column thermostat and controlled by Chem-Station software. The Symmetry C18 (250 × 4.6 mm), 5µm was used as a stationary phase.

Chemicals and reagents
The reference standard of Venlafaxine hydrochloride sustained release tablets was obtained from Analytical services department of strides Arcolab Ltd. Bangalore, India. Venlafaxine hydrochloride sustained release tablets were in-house product of formulation development department, strides Arcolab Ltd. Bangalore, India. All solvents were used HPLC grade and reagents also, like Acetonitrile, Potassium dihydrogen phosphate, N, N-dimethyl octylamine, ortho phosphoric acid, Hydrochloric acid and HPLC grade water was obtained by passage through a Milli-Q system

General Equipments:

The chromatographic column used was 250 mm × 4.6 mm, water symmetry C18, with 5µm particles. The flow rate of the mobile phase was maintained at 0.8 ml/min and the column temperature 30°C. Detection was carried out at 225 nm and the injection volume was 10 µL. Run time was 3.5 times the retention time of Venlafaxine.

Mobile phase preparation and Standard preparation

Buffer Solution:

Dissolve 4.08 gram of potassium dihydrogen phosphate in 1000 mL of water, add 1 mL of N,N-dimethyl octylamine and adjust pH to 2.8 with ortho phosphoric acid.

Mobile Phase Preparation:

Mix 80 volumes of buffer solution and 20 volumes of Acetonitrile. Filtere through 0.45 µm membrane filter and degas.

Preparation of standard solution:

Weigh accurately and transfer about 25 mg of Venlafaxine hydrochloride standard into a 100 mL volumetric flask. Dissolve and make up with mixture of water and Acetonitrile in the ratio of 50:50.

Further, dilute 1.0 mL of this solution to 250 mL with the mobile phase to obtain a concentration of 0.001 mg/mL.

Preparation of blank solution:

Dilute 5 mL of 0.1 N HCl to 10 mL with mobile phase.

Preparation of sample solution:

 Weigh accurately and transfer 50 mg of powdered tablets into a 100 mL volumetric flask. Add about 50 mL of diluent.

Sonicate for 10 minutes and then place it on a water bath for 30 minutes (temperature 75°C) with occasional shaking. Make up to the mark with mobile phase. Filter through 0.45µ filter.

The above solutions were injected at above chromatographic conditions and peak areas were measured to determine the related impurities of Venlafaxine in the drug product Venlafaxine hydrochloride sustained release tablets.

RESULTS AND DISCUSSION

Method for Related Impurities

This method is used for the quantitative determination of related impurities of Venlafaxine in the drug product Venlafaxine hydrochloride sustained release tablets, different mobile phases, stationary phases were employed, and the proposed chromatographic conditions were found appropriate.

System suitability results are as follows.

Ratio between the areas of Venlafaxine HCl from the duplicate injections of Reference solution 0.99

Resolution between amine impurity and Venlafaxine is 4.021

Theoretical plate for the Venlafaxine peak is 9800.

The retention time of Venlafaxine hydrochloride is 7.624 minutes.

Method Validation

The proposed method was validated for estimation of related impurities of Venlafaxine in the drug product Venlafaxine Hydrochloride sustained released tablets using following parameters.

Specificity

To demonstrate the specificity, potential contaminants were generated by forced degradation. The chromatograms were taken on photo diode array detector and the peak purities were found to be 0.99 to 1.

Linearity

Linearity was studied by preparing standard solutions at different concentration levels of impurities and venlafaxine. When the concentrations of impurities, Venlafaxine and its respective peak responses were subjected to regression analysis by least squares method, a good linear relationship (r = 0.9999) was

observed between the concentrations of impurities, venlafaxine and the respective peak responses in the range LOQ to 300% level of unknown and known impurity specification limits. The regression equation was found to be Y = 23.45 x + 125.36, where Y is the peak area and X is the concentration of impurity.

Limit of Detection and Limit of Quantitation:

LOD was defined as $3.3\sigma/S$ and LOQ was $10\sigma/S$ based on 'standard deviation of the response and slope of the calibration curve specially constructed in a low region of the target analyte concentration. The standard deviation of y-intercepts of the regression lines was used as σ (the standard deviation of the response) and 'S' is the slope of the calibration curve. The LOD and LOQ were found to be 0.26 and 0.81 ppm, respectively.

Method accuracy

To ensure the reliability and accuracy of the method, recovery studies were carried out in triplicate at four concentration levels (50%, 100%, 200% and 300%) of impurity specification level. The recovery of impurities and Venlafaxine was found to be in the range of 96.0-100.0 %

Table I: Accuracy

Level (%)	mg added	mg recovered	%Recovery Mean (n=3)
50 %	0.0379	0.0406	98.1
100 %	0.0757	0.0730	96.4
200 %	0.1515	0.1484	98.0
300 %	0.2272	0.2261	99.5

Precision study

The intra-day precision of the related impurities of Venlafaxine method was evaluated by carrying out six independent Venlafaxine hydrochloride tablets test samples against qualified reference standard on same day and these studies were repeated on six consecutive days to determine inter-day precision. The percentages of RSD of six know/unknown impurities values were calculated. Results are as follows.

Table II: Precision (Summary of precision study)

Study	Desmethyl Impurity	Hydroxy Methyl Impurity	Amine Impurity	Dehydrated Impurity	unknown Impurity	Total Impurities
Intra Day precision	0.03	0.05	0.08	0.03	0.04	0.26
Inter Day precision	0.02	0.06	0.07	0.03	0.03	0.21
% RSD	3.8%	4.2%	2.1%	2.3%	1.9%	3.2%

Above all values are mean of six (n=6).

Standard and sample solution stability

The solution stability of venlafaxine standard and test solution was carried out by leaving the solutions in a tightly capped volumetric flask at room temperature for 48 hours. After 48 hours completion impurities are evaluated freshly prepared solutions. The relative standard deviation was found below 5.0 % for all impurities in test solution and standard solution ratio is 0.99. It

showed that both standard and sample solutions were stable up to 48 hours at room temperature.

Method robustness

This was done by small deliberate changing in flow rate, pH of mobile phase, mobile phase ratio and column oven temperature. Results are shown. Results show that the contents of the drug were not adversely affected by these changes as evident from the low value of ratio indicating that the method was robust.

Table III: Robustness (Summary of Robustness results)

The difference factor of two standard injections area response should be between 0.95 and 1.05.				
Original Condition	Decrease in flow rate(0.6ml/min)	Increase in flow rate(1.0ml/min)	Decrease in column Temperature(20°C)	Increase in column Temperature(30°C)
0.99	1.00	0.99	0.99	1.00

Method Ruggedness

Ruggedness test was determined between two different analysts, instruments and columns. Results are shown in below table

Table IV: Ruggedness

	Desmethyl Impurity	Hydroxy Methyl Impurity	Amine Impurity	Dehydrated Impurity	Unknown Impurity	Total Impurities
Analyst1,column 1, instrument 1	0.03	0.04	0.05	0.03	0.05	0.26
Analyst 2,column 2, instrument 2	0.02	0.05	0.06	0.02	0.04	0.21
% RSD	1.8%	2.0%	2.9%	4.2%	3.6%	2.1%

Conclusion:

Analytical RP-HPLC method was developed and validated for the determination of related impurities in the drug product Venlafaxine hydrochloride sustained release tablets. The developed method was found to be simple, precise and accurate and can be applicable for the routine quality control analysis of related impurities of venlafaxine hydrochloride in the drug product Venlafaxine hydrochloride sustained release dosage form. The advantages of the method are simplicity of sample preparation, no need of derivative formation, which

require longer time for analysis. As a result, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of
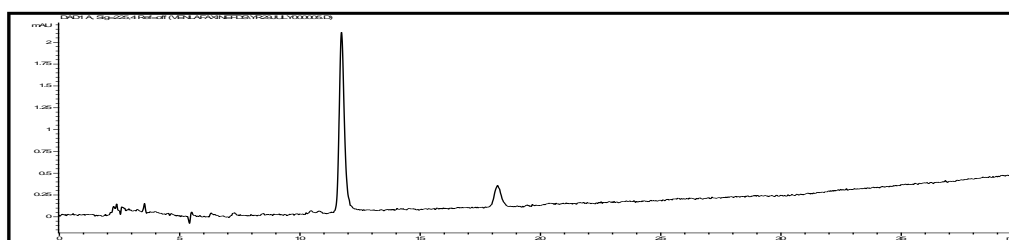
column and post column flushing; that are typically associated when different chromatographic conditions are used.

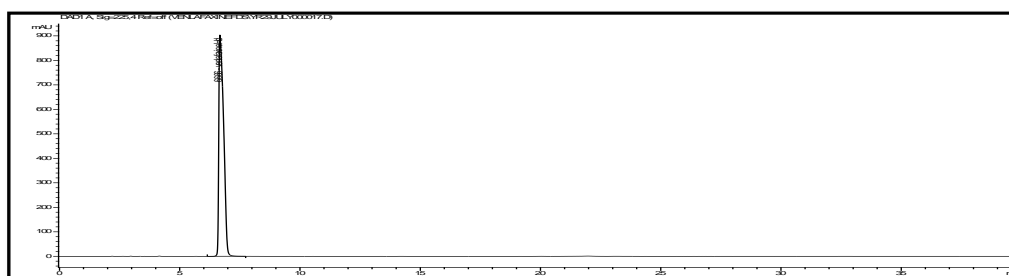**Chromatograms References:**

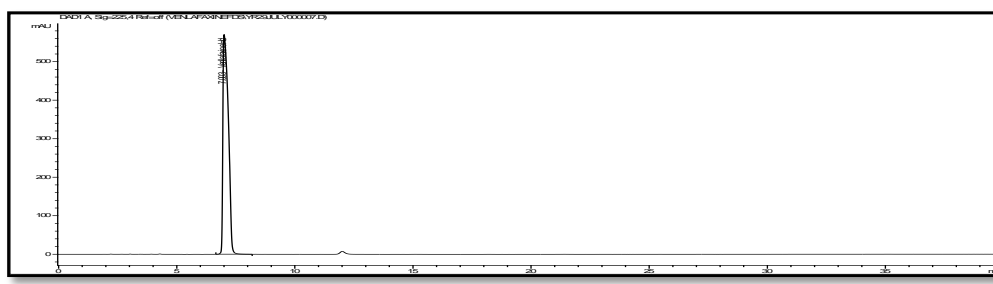Chromatogram No I: Blank preparation
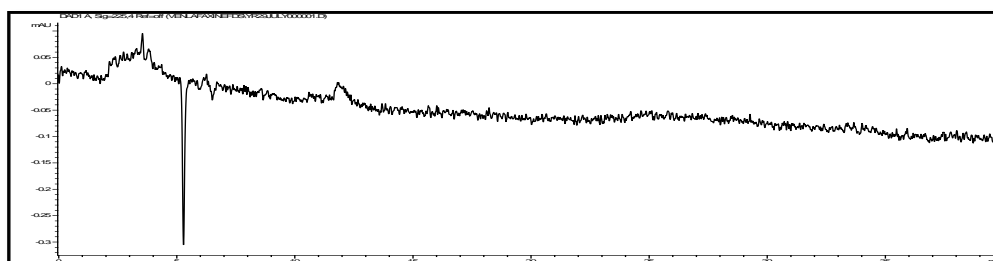
Chromatogram No II: Placebo preparation
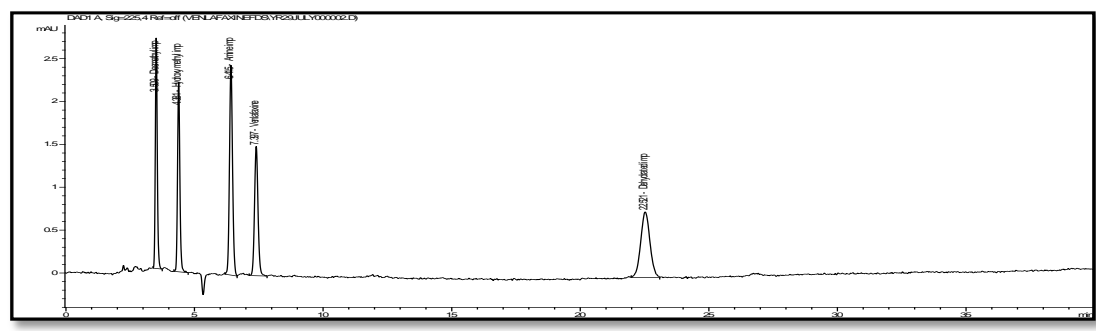

Chromatogram No III: Reference standard preparation

Chromatogram No IV: Sample preparation

Chromatogram No V :   Mixture of Impurities and Venlafaxine HCl

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