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Research Article

New Chiral Normal Phase UFLC Method for Determination of Venlafaxine Hydrochloride Enantiomers in Pharmaceutical Formulations

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ABSTRACT

A simple, specific, precise, sensitive and rapid normal phase-Ultra Fast Liquid Chromatography (NP-UFLC) method was developed for determination of venlafaxine hydrochloride enantiomers in pharmaceutical formulation. The method was developed on a Lux amylase 2 column (150 x 4.0 mm I.D., particle size 5 µ) with the mobile phase was n-hexane and ethanol (97:3 v/v); in 0.1 % diethyamine using UV detector was fixed at 254 nm with a flow rate was 1 ml/ min. The retention time (t_R) of both enantiomer were found to be 4.1±0.2 min and 4.8±0.3 min, respectively. The linearity over the concentration range of 5-30 µg/ ml for venlafaxine. The intra-day and inter-day coefficient of variation of the assay method were found to be 0.293 to 1.760 and 0.319 to 0.210 respectively for enantiomer 1 and 2, with high accuracy and precision results. The proposed NP-UFLC method is suitable for analysis of venlafaxine hydrochloride enantiomers in pharmaceutical dosage forms. The validated NP-UFLC method was developed for the quantification of venlafaxine in tablet dosage form.

Keywords: Venlafaxine hydrochloride, Enantiomers, Chiral drug separation, NP-UFLC, Validation.

INTRODUCTION

Venlafaxine hydrochloride chemically known as, Cyclohexanol 1-(2-(dimethylamino)-1-(4-methoxyphenyl)ethyl)- hydrochloride (Fig. 1) is a second-generation antidepressant drug marketed as a racemic mixture. It is an antidepressant of the serotonin-norepinephrine reuptake inhibitor (SNRI) class[1]. Venlafaxine increases the concentrations of the neurotransmitters serotonin and norepinephrine in the body and the brain[2,3]. The R-enantiomer shows dual presynaptic inhibition of serotonin and noradrenaline uptake, whereas the S-enantiomer is a serotonine reuptake inhibitor. Thus, the drug is the first and most commonly used serotonin and noradrenaline reuptake inhibitor. Its synthesis along with that of several analogues were described many years ago. The synthetic routes are similar and vary according to the nature of the aromatic substituents. However, the final products are racemic mixtures, and they were crystallized as hydrochlorides[4]. Although the disposition of venlafaxine in humans was originally found not to be stereoselective[5]. In view of the near expiration date (June 2008) of the first patent for the racemic compound and of these recent clinical findings, venlafaxine appears to be a good candidate for a chiral switch[6-8]. The trend toward single enantiomer drugs is clear and the number of racemic drugs that reach the market as new chemical entities is decreasing[9]. The relevance of chirality in antidepressant drugs was highlighted several years ago and many examples are illustrated in a recent very complete review[10,11]. In the previously cited research on the resolution of venlafaxine, the enantiomers were separated by either of two general approaches. The first is the classical method of diastereoisomeric salt formation and fractional crystallization and the second approach uses analytical enantioselective electro driven methods. In the latter cases, either cyclodextrinsin capillary electrophoresis[12]. There is only one literature report where an HPLC baseline separation of the enantiomers of venlafaxine extracted was achieved using a CSP and normal phase

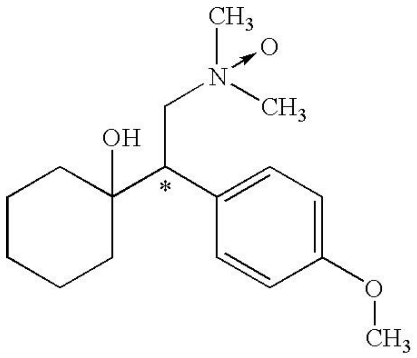

Figure 1: Venlafaxine Hydrochloride.

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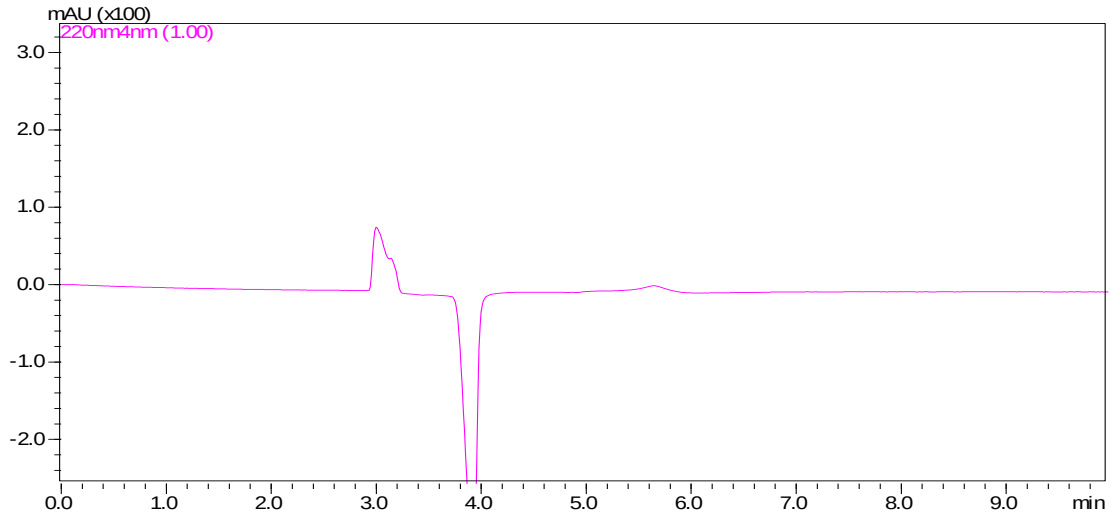

Figure 2: Blank chromatogram.

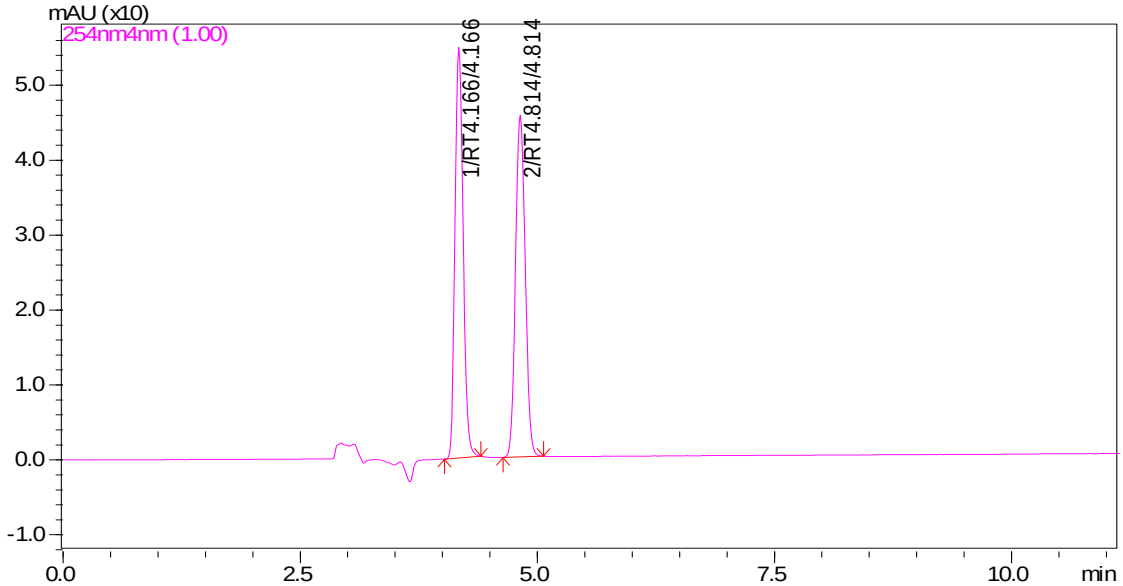

Figure 3: Standard chromatogram for both the enatiomers of venlafaxine hydrochloride.

Table 1: System suitability parameters and linearity data of enantiomer-1 and enantiomer-2.

Parameters	Enantiomer-1	Enantiomer-2
Retention time (min)	4.1±0.2	4.8±0.3
Plate count	9175	6894
Tailing factor	1.1	0.8
Resolution	--	3.664
Beer's range (µg/ml)	5-30	5-30
Slope (m)	10823	10581
Y Intercept	3282.6	1542
Correlation coefficient (r^2)	0.997	0.997
LOD (µg/ml)	0.06	0.05
LOQ (µg/ml)	0.20	0.197

mode[13]. From an analytical point of view, enantioselective chromatography offers the advantages of a method that can be developed on a semipreparative or preparative scale for the isolation of single enantiomers, which then become available for pharmaceutical testing strategies and requirements for enantioselective[14]. In the present research work, a simple, sensitive and accurate normal phase UFLC method to separate both the enantiomer of venlafaxine in bulk drugs and tablets using Lux amylase 2 column column has been reported for first time. The method was also validated to ensure the compliance in accordance with the ICH guidelines.

MATERIALS AND METHODS

Chemicals and Reagents

Venlafaxine hydrochloride enantiomers were procured as a gift sample from RL Fine Chemicals Bangaluru, India. The solvents like n-hexane and ethanol diethylamine used was of HPLC grade (Merck, India). Commercially available racemic venlafaxine hydrochloride tablets claimed to contain 25 mg of drug were procured from local pharmacy.

Instrumentation

Quantitative NP-UFLC was performed on gradient high pressure liquid chromatography (Shimadzu) auto sampler consisting of a LC–20HT solvent module, SPD– 10A, and an PDA detector with LC software. The column

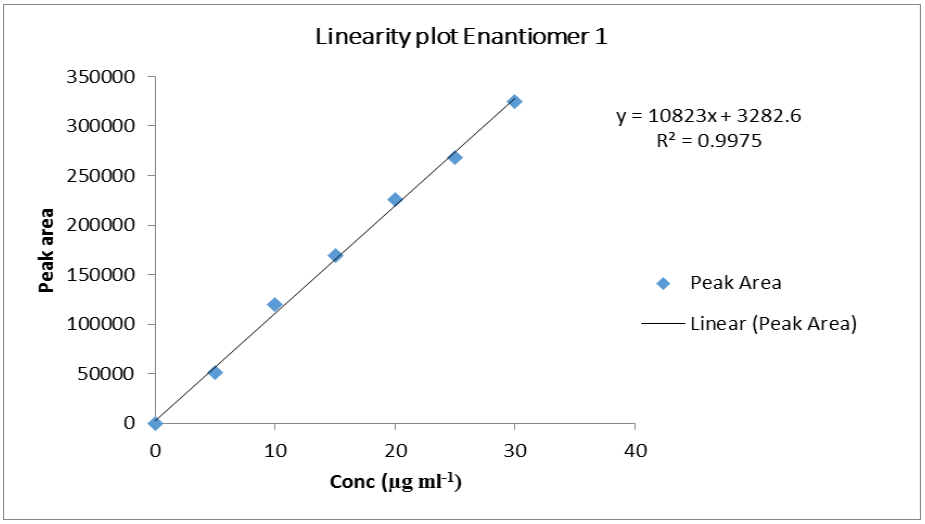

Figure 4: Linearity plot for enatiomers 1 of venlafaxine hydrochloride.

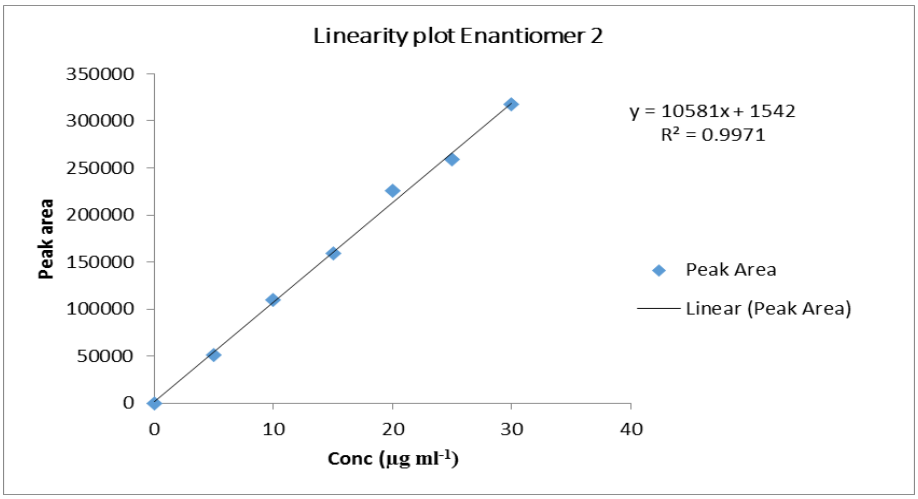

Figure 5: Linearity plot forenatiomers 2 of venlafaxine hydrochloride.

Table 2: Accuracy data of enantiomer-1 and enantiomer-2.

Sample	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	% RSD*
Enantiomer-1	5	4.941	98.82	1.149
	10	10.096	100.96	0.405
	15	15.062	100.41	0.193
Enantiomer-2	5	5.080	101.6	1.423
	10	10.09	100.9	1.983
	15	14.931	99.54	0.652

*Mean value of six determinations

used was LUX amylase 2 chiral column(150 x 4.0 mm) particle size 5 µ.

Mobile phase

n-Hexane and ethanol of HPLC grade was taken as mobile phase in the ratio of 97:3 % (v/v).

Preparation of standard stock solution

Standard stock solution (100 µg/ml) of Venlafaxine hydrochloride was prepared by weighing exactly 10 mg of drug dissolved in isopropanol and diluted to 100 ml with same solvent.

UFLC conditions

The composition of the mobile phase was n-hexane and ethanol in the ratio of 97:03 v/v. They were filtered before use through a 0.2 mm membrane filter, degassed in a bath sonicator for 10 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1 ml min^{-1}, which yielded a column backpressure of 96 kg/cm^2. The run time was set at 20 min and column temperature was ambient. The volume of injection loop was 20 ml. prior to injection of drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The

Table 3: Precision data of enantiomer-1 and enantiomer-2

Theoretical concentration (µg/ml)	Intraday(n=6) (µg/ml)	%RSD	Inter day(n=6) Found(µg/ml)	%RSD
Enantiomer-1				
5	5.008	1.003	5.052	0.276
10	10.047	0.293	10.059	1.766
15	15.092	0.119	15.092	0.122
Enantiomer-2				
5	5.064	1.524	5.078	1.002
10	10.078	0.319	10.020	0.210
15	15.092	0.119	15.092	0.122

Table 4: Assay of Venlafaxine.

Sample	Injected (µg/ml)	Amount found (µg/ml)	% found	Amount	%RSD
Enantiomer-1 (EFFEROX 25mg tablet)	10	10.047	100.47		0.286
Enantiomer-2 (EFFEROX 25mg tablet)	10	9.978	99.78		0.329

Table 5: Robustness data of enantiomer-1 and enantiomer-2.

Parameters	%RSD
Flow Rate	1.45
0.98 ml/min	
1.02 ml/min	
Wavelength (nm)	0.838
250	
258	
Mobile phase composition	0.914
97:3	
99:1	

eluents were monitored at 254 nm and data was acquired, stored and analyzed with the LC 10 software.

Preparation of calibration curve

Aliquots of Venlafaxine hydrochloride ranging from 0.5-3 ml (each ml contains 100 µg/ ml) were pipetted into as a series of 10 ml volumetric flasks. The volume was made up to the mark with isopropanol. Aliquoets of 10 µL was injected (six time) into HPLC. The elution of the drug measured at 254.0 nm. The amount of venlafaxine hydrochloride present in the sample solution was computed from its calibration curve and it was constructed by plotting peak area of chromatogram against the concentration of Venlafaxine hydrochloride. The blank chromatogram and standard drug chromatogram were recorded respectively. Linearity plot for concentration of 5.0-30 µg/ ml for both the enatiomers of venlafaxine hydrochloride was constructed.

Analysis of tablet dosage form

Five tablets (**EFFEROX)**, each containing 25 mg of venlafaxine hydrochloride were weighed and crushed into fine powder form. Powder equivalent to 125 mg of venlafaxine hydrochloride was weighed and transferred to a standard volumetric flask. The contents were mixed thoroughly with isopropanol, then diluted with same solvent. The resulting solution was filtered through 0.45 µm membrane filter and diluted to the required concentration and 10 µl of the sample was injected on to UFLC system for the analysis of assay of the drug content.

RESULTS AND DISCUSSION

Validation of the method

The developed method for the assay of venlafaxine has been validated as per the current ICH Q2 (R1) guidelines[15].

Analytical parameters

The development of NP-UFLC method for the determination of enantiomers has received a considerable attention in recent past because of its importance in the quality control of drugs and drug products. The assay of venlafaxine hydrochloride enantiomers was resolved with good accuracy. The retention time (t_R) of both the enantiomer were found to be 4.1±0.2 min and 4.8±0.3 min, respectively. The blank chromatogram and a typical chromatogram of standard Venlafaxine hydrochloride were shown in Fig 2 and 3 respectively. Tailing factor for both venlafaxine hydrochloride (Enantiomer 1) and venlafaxine hydrochloride (Enantiomer 2) were found to be 1.1 and 0.8 respectively.

The calibration curve was constructed by plotting the peak areas against the concentration of both the enantiomer (1 and 2) of venlafaxine hydrochloride in 5-30 µg/ml as shown in the Fig. 4 and 5 respectively. It was found to be linear with a correlation coefficient of 0.9975 for Enantiomer 1 and 0.9971 for Enantiomer 2 of venlafaxine hydrochloride, the representative linear regression equation being y = 10832X + 3282.6 and y = 10581X + 1542 for both the enantiomers respectively. The slope, y-intercept, and their standard deviations evaluated are presented in Table 1.

System suitability

The system suitability was studied under validation parameter by injecting six replicates of the standard solution of venlafaxine hydrochloride. Adequate resolution of >2 between R-and S- isomer of venlafaxine hydrochloride demonstrated the method specificity in the presence of its synthetic precursor, as shown in Figure 3.

Accuracy

The amount of venlafaxine hydrochloride enantiomers in the matrix was calculated using following formula.

% Recovery = T-A /S×100

Where, T is total amount of drug estimated, A is the initial amount of drug in the tablet powder and S- amount

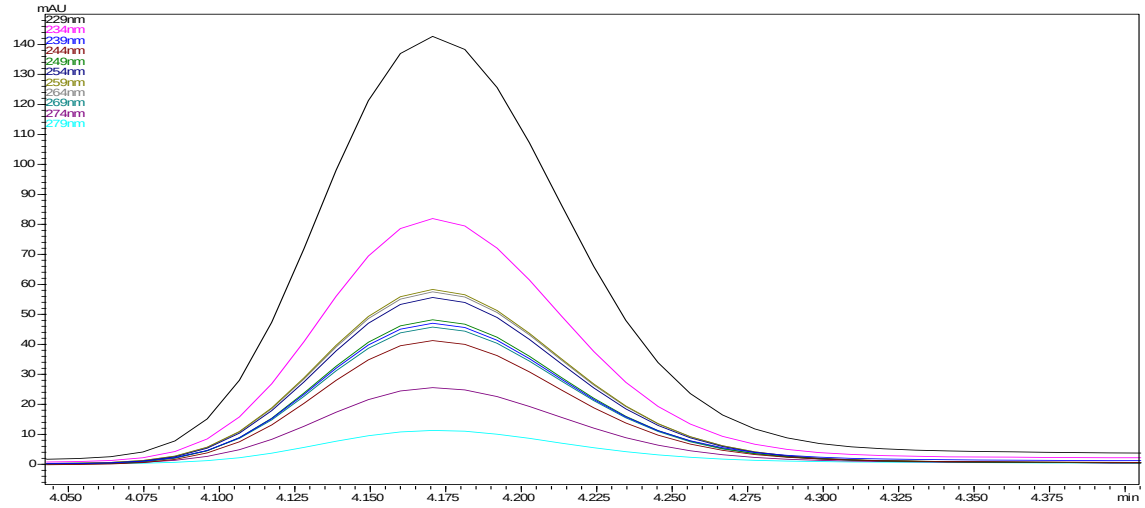

Figure 6: Peak profile for enatiomers 1 of venlafaxine hydrochloride.

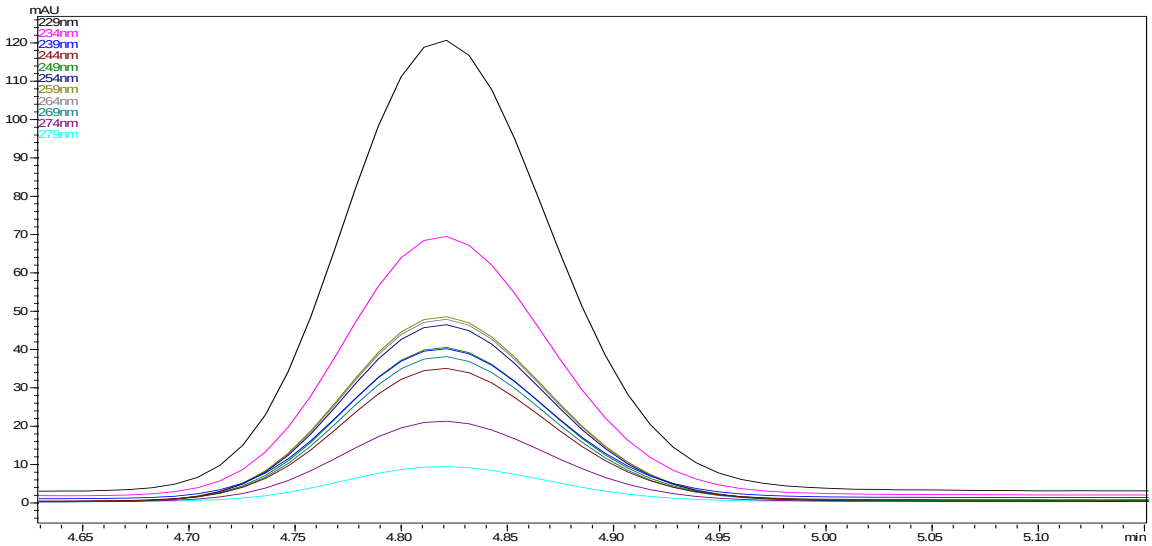

Figure 7: Peak profile for enatiomers 2 of venlafaxine hydrochloride.

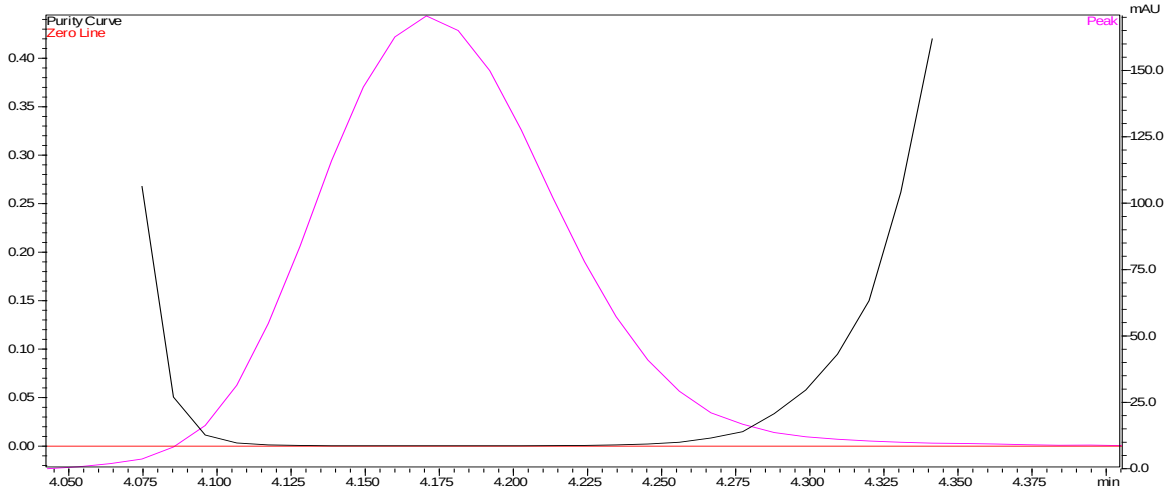

Figure 8: Peak purity for enatiomers 1 of venlafaxine hydrochloride.

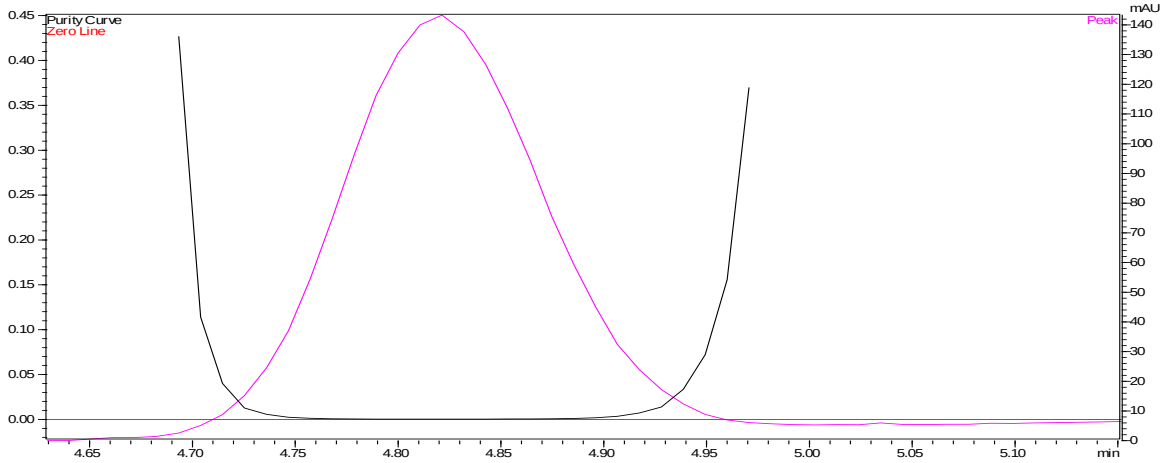

Figure 9: Peak purity for enatiomers 2 of venlafaxine hydrochloride.

of pure drug added. The results shown in Table 2 revealed, high recovery of venlafaxine hydrochloride enantiomers, indicating that the proposed method for the determination of venlafaxine hydrochloride enantiomers in the tablet is highly accurate.

Precision

Method precision was determined both in terms of repeatability (injection and analysis) and intermediate precision (intra-day and inter-days reproducibility). The values are summarized in Table 3. These values were within the standard limits.

Limit of detection and limit of quantification

Limit of detection and limit of quantification can be calculated using the following equation according to ICH guidelines:

LOD = 3.3 x N/S

LOQ = 10 x N/S

where N is the standard deviation of peak areas of the drug and S is the slope of the corresponding calibration curve. The results are shown in Table 1.

Assay of the drug

The chiral NP-HPLC method developed in the present investigation was used to quantify venlafaxine hydrochloride enantiomers in tablet dosage forms. The obtained results are given in Tables 4. The average drug content was found to be 10.047 mg for enantiomer 1 venlafaxine hydrochloride and 9.978 mg for enantiomer 2 venlafaxine hydrochloride of the labelled amount in 25mg of racemic venlafaxine hydrochloride, respectively. The peak profile and peak purity of both enantiomers are shown in Fig. 6, 7, 8 and 9.

Robustness of the method and stability of the solution

The robustness of an analytical procedure has been defined by the ICH as a “measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. The most important aspect of robustness is to develop methods that develop methods that allow for expected variations in method parameters. According to ICH guidelines, robustness should be considered early in the development stage of a method. The typical variations studied under this parameter are flow rate, wavelength and mobile phase composition. The results are tabulated in Table 5.

CONCLUSION

A precise, accurate, robust, and specific NP-UFLC method has been developed and validated for the enantiomeric separation of venlafaxine in tablet formulation. The agreeable results were obtained from the validation of the method. The short retention time (4.1 min for enantiomer 1 and 4.8 min for enantiomer 2) obtained provides rapid determination of venlafaxine, which is significant for its routine analysis in quality control. The method exhibits an excellent performance in terms of sensitivity and robust. The experimental results of the present study showed that the proposed NP-UFLC method is simple, specific, precise, sensitive, rapid and accurate and is useful for separation of venlafaxine hydrochloride enantiomers in its pharmaceutical formulation.

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