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# Stability indicating LC method for the estimation of venlafaxine in pharmaceutical formulations

Sapna N. Makhija, Pradeep R. Vavia *

*Pharmaceutical Division, University Department of Chemical Technology (Autonomous), University of Mumbai, Nathalal Parikh Marg, Matunga, Mumbai 400019, India*

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**Abstract**

A rapid, selective and stability indicating high performance liquid chromatographic method was developed and validated for the estimation of venlafaxine in pharmaceutical dosage forms. The analysis was done on a Spherisorb C8 (4.6 × 250 mm, 5 μm) column. The mobile phase consisted of acetonitrile:sodium dihydrogen orthophosphate [0.04 M], pH 6.8 (75:25) at a flow rate of 1.5 ml/min. Detection was carried out at a wavelength of 224 nm. The developed method was found to give good separation between the pure drug and the degraded product. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 1–10 μg/ml with $r = 0.9999$. The method was validated for precision, accuracy, ruggedness and recovery. The minimum detectable and minimum quantifiable amounts were found to be 150 and 600 ng/ml, respectively. The drug was stable under basic and oxidative conditions. However, the sample treated with acid showed an additional peak at a retention time of 4.32 min other than the main peak at a retention time of 5.32 min. Statistical analysis proves that the method is reproducible and selective for the estimation of venlafaxine. As the method could effectively separate the drug from the degradation product, it can be employed as a stability indicating one. © 2002 Published by Elsevier Science B.V.

*Keywords:* Venlafaxine; HPLC; Stability indicating

## 1. Introduction

Venlafaxine, 1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol hydrochloride is a novel, non-tricyclic antidepressant. Venlafaxine imparts its antidepressant effects by inhibiting the neuronal uptake of norepinephrine, serotonin and to a lesser extent, dopamine. [1–5]. It lacks monoamine oxidase activity and, more importantly, lacks the adverse effect profile of tricyclic antidepressants [4,5]. Venlafaxine has no affinity for brain muscarinic, cholinergic, histaminergic or α adrenergic receptors [5,6]. Various HPLC methods [7,8] are reported in literature for the analysis of venlafaxine. However, none of these methods are stability indicating.

An ideal stability indicating chromatographic method should estimate the drug and also be able

* Corresponding author. Tel.: +91-22-414-5616; fax: +91-22-414-5614.
*E-mail address:* prv@pharma.udct.ernet.in (P.R. Vavia).

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to resolve the drug from its degradation products. Hence, an attempt has been made to develop an accurate, rapid, specific and reproducible method for the determination of venlafaxine in the presence of the degradation products for the content analysis during stability studies from pharmaceutical dosage forms.

## 2. Experimental

### 2.1. Materials

Venlafaxine hydrochloride was obtained from M/s. Alembic Ltd., Baroda, India. All other solvents and reagents were purchased from Ranbaxy chemicals, India and were of analytical grade.

### 2.2. Instrumentation

The chromatograph system comprised of a Jasco PU-980 pump equipped with a Jasco UV-975 detector. Data integration was done using a Borwin software package V1.21. The column used was Spherisorb C8 ($4.6 \times 250$ mm, 5 μm). Injections were carried out using a 20 μl loop at 25 °C.

### 2.3. Stability indicating method

The drug was subjected to forced degradation under acidic conditions (1 M HCl), basic conditions (1 M NaOH) and oxidation ($H_2O_2$) by heating at 70 °C for 1 h. A 20 mg/ml aqueous solution was prepared and accordingly treated. These solutions were further neutralised, diluted to a final concentration of 30 μg/ml with the mobile phase and injected.

### 2.4. Optimisation of solvent system

Varying compositions of acetonitrile:sodium dihydrogen orthophosphate [0.04 M], pH 6.8 viz. 25:75, 40:60, 50:50, 75:25, v/v were evaluated as mobile phase in order to achieve good resolution between the drug and its degraded product.

### 2.5. Method validation

The developed method was validated for linearity, accuracy, precision, recovery, limit of detection, limit of quantification and ruggedness.

#### 2.5.1. Linearity

Stock solution of venlafaxine hydrochloride (1 mg/ml) was prepared in mobile phase. A series of standard curves were prepared over a concentration range of 1–10 μg/ml. The data of peak area versus drug concentration was treated by linear least square regression analysis. The standard curves were evaluated for intra-day and inter-day reproducibility.

#### 2.5.2. Precision and accuracy

A standard solution of 6 μg/ml was selected and analysed six times.

#### 2.5.3. Recovery

The analysed samples were spiked with 50, 100 and 150% of the standard drug and the mixtures were reanalysed by the proposed method ($n = 3$). The extraction solvent employed was methanol.

#### 2.5.4. Limit of detection and limit of quantification

In order to estimate the limit of detection and limit of quantification, mobile phase was injected six times. The noise level was determined. The limit of detection was calculated to be three times the noise value and ten times the noise gave limit of quantification.

#### 2.5.5. Ruggedness

The ruggedness of the proposed method was studied using reagents from different lots and different manufacturers.

#### 2.5.6. Stability of analyte

A solution of 6 μg/ml was analysed at intervals of 0, 24, 48 and 72 h, storing the samples at room temperature. Relative standard deviation of data gave an estimate of the stability of the analyte.

*2.5.7. Analysis of the developed formulation*

To determine the content of venlafaxine from the tablets (label claim: venlafaxine hydrochloride equivalent to venlafaxine base 75 mg per tablet), 20 tablets were powdered and about 100 mg of powder equivalent to 18 mg of venlafaxine was weighed accurately and transferred to a 25 ml volumetric flask. Methanol was used for extraction. To ensure complete extraction of the drug it was sonicated for 20 min and the solution was made upto 25 ml. The resulting solution was centrifuged and the supernatent was diluted with the mobile phase and injected. The analysis was repeated in triplicate. A placebo tablet was also subjected to the same extraction process as discussed above and injected. The possibility of excipient interference in the analysis was studied.

## 3. Results and discussion

### *3.1. Optimisation of solvent system*

When acetonitrile:sodium dihydrogen orthophosphate [0.04 M], pH 6.8 in the proportions 25:75, 40:60, 50:50, v/v was employed as the mobile phase, poor resolution was obtained between the drug and its degradation product. The mobile phase acetonitrile:sodium dihydrogen orthophosphate [0.04 M], pH 6.8, 75:25 was found to be a suitable solvent system. The drug was eluted at a retention time of 5.32 min and the degraded product at a retention time of 4.32 min.

### *3.2. Stability indicating method*

The drug does not degrade under basic and oxidative conditions as the chromatograms of the base and hydrogen peroxide treated samples showed only the peak of the pure drug (retention time 5.32 min). The acid degraded sample showed an additional peak at a retention time of 4.32 min (Figs. 1–3). The hypothetical acid degraded product for venlafaxine would result by the *o*-demethylation of the 4-methoxy group attached to the phenyl ring (Fig. 1). This compound being more polar in nature is eluted faster.

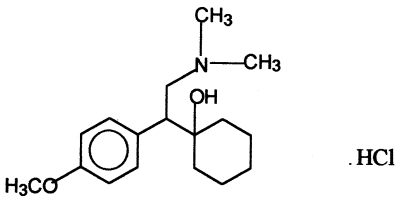


Fig. 1. Structure of venlafaxine hydrocloride.

### *3.3. Validation*

*3.3.1. Linearity*

The polynomial regression data for the calibration plots ($n = 3$) showed a good linear relationship over a concentration range of 1–10 μg/ml. The coefficient of correlation was $0.9999 \pm 0.0012$ with slope and intercept values $24\,593.03 \pm 234.99$ and $155.19 \pm 1.38$, respectively. No significant difference was observed in the slopes of standard curves (ANOVA; $P > 0.05$).

*3.3.2. Precision and accuracy*

The results in Table 1 revealed excellent accuracy and high precision of the assay method.

*3.3.3. Recovery*

The proposed method when used for extraction and subsequent estimation of the drug from pharmaceutical dosage forms after spiking with 50, 100 and 150% of additional drug afforded recovery of 98–102% as listed in Table 1.

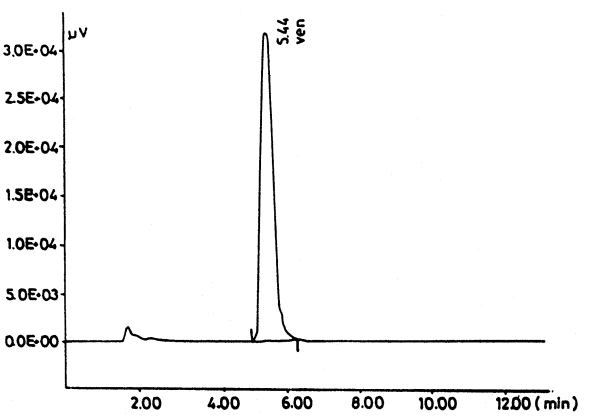


Fig. 2. Chromatogram for standard venlafaxine (ven), 30 μg/ml.

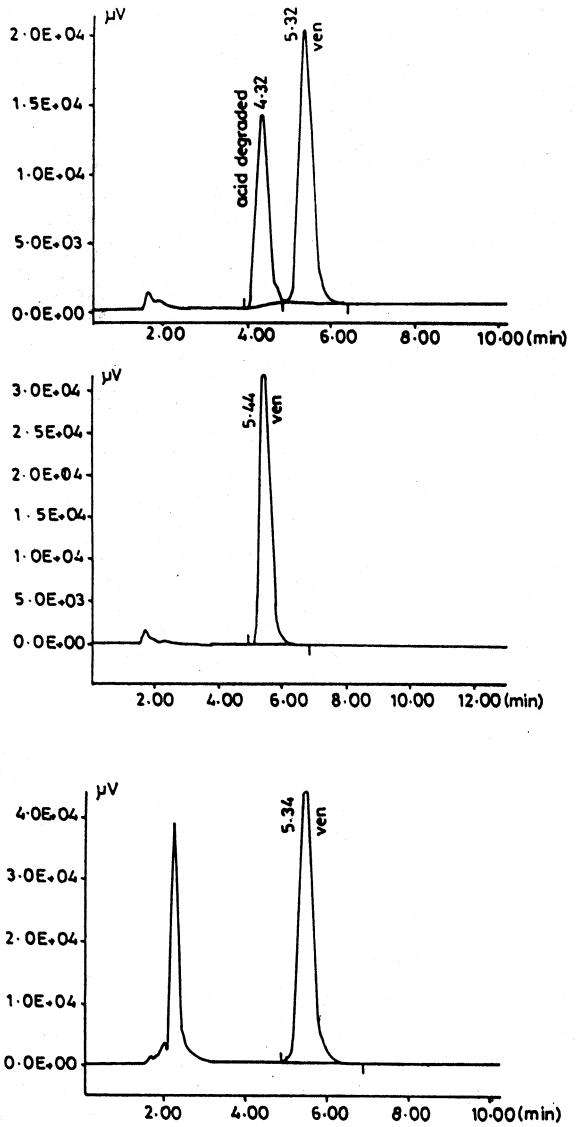


Fig. 3. Chromatograms for acid degraded, base degraded and oxidised samples of venlafaxine (ven).

### 3.3.4. Limit of detection and limit of quantification

The minimum detectable amount with a signal to noise ratio of 3:1, was found to be 150 ng/ml. The limit of quantitation, with a signal to noise ratio of 10:1, was found to be 600 ng/ml.

### 3.3.5. Ruggedness

The R.S.D. for system precision and accuracy studies was found to be 1.18 and 1.10% for different lots of reagents and 1.30 and 1.44% for different manufacturers, respectively.

### 3.3.6. Stability of analyte

The analyte was found to be stable since no significant deviation in the peak areas was observed on analysis even after 72 h with the R.S.D. value being 0.79%.

### 3.4. Analysis of the formulation

The drug content was found to be within the limits as evidenced from Table 1. The formulation was a sustained release tablet based on hydrophilic matrix system i.e. hydroxypropylethylcellulose. Other excipients included diluent like microcrystalline cellulose and lubricants like purified talc and magnesium stearate. There was no interference from the excipients present in the tablet.

## 4. Conclusion

The developed HPLC technique is precise, specific, accurate and stability-indicating. The statis-

Table 1
Validation method

| Tested concentration (µg/ml) | Mean ± S.D. | R.S.D. (%) |
|---|---|---|
| *Accuracy* | | |
| 6 | 145 861.69 ± 2245.28 | 1.54 |
| *Precision* | | |
| 6 | 144 879.19 ± 1448.37 | 0.99 |
| Theoretical content (mg) | | |
| *Recovery* (%) | | |
| 112.50 | 99.96 ± 1.22 | 1.23 |
| 150.00 | 102.08 ± 1.39 | 1.37 |
| 187.50 | 101.71 ± 1.92 | 1.89 |
| *Drug content* (%) | | |
| 75 | 101.82 ± 1.03 | 1.01 |

tical analysis proves that the method is reproducible and selective for the estimation of venlafaxine in pharmaceutical formulations. As the method could effectively separate the drugs from the degradation product it can be employed as a stability indicating one.

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## References

[1] M. Rudorfer, W. Potter, Drugs 37 (1989) 713–738.
[2] J. Moyer, E. Muth, J. Haskins, R. Lappe, E. Sigg, Soc. Neurosci. 10 (1984) 261–262.
[3] S. Sisenwine, J. Politowski, K. Birk, G. White, M. Dyroff, Acta Pharmacol. Toxicol. 59 (5) (1986) 312.
[4] J. Haskins, J. Moyer, E. Muth, E. Sigg, Eur. J. Pharmacol. 115 (1985) 139–146.
[5] E. Muth, J. Haskins, J. Moyer, G. Husbands, S. Nielsen, E. Sigg, Biochem. Pharmacol. 35 (1986) 4493–4497.
[6] J. Pento, Drugs Future 13 (1988) 839–840.
[7] D. Hicks, D. Wolaniuk, A. Russell, N. Cavanaugh, M. Kraml, Ther. Drug Monitor. 16 (1994) 100–107.
[8] R. Vu, D. Helmeste, L. Albers, C. Reist, J. Chromatogr. B 703 (1997) 195–201.