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Research Article

New, sensitive and validated spectrofluorimetric method for the estimation of desvenlafaxine succinate in bulk and in pharmaceutical formulations

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ABSTRACT

A New, simple, sensitive and economical spectrofluorimetric method for estimation of Desvenlafaxine Succinate, a novel SNRI (serotonin - norepinephrine reuptake inhibitor) in bulk and in solid dosage form was developed in the present study. Desvenlafaxine Succinate showed excitation at 274 nm and emission at 305 nm in pH=6 phosphate buffer which was used in this study. The linear regression equations obtained by least square regression method, was Int =0.396*Conc. (in ng/ml) -1.701. The method provides a linear response across a quantization range of 100ng/ml to 900ng/ml. The developed method was found to have high degree of precision and accuracy and was used for the estimation of total drug content in the commercial formulation of Desvenlafaxine Succinate-ER tablets. The results of analysis were treated statistically, as per International Conference on Harmonisation (ICH) guidelines for validation of analytical procedures. The results were found to be accurate, reproducible and free from interferences.

Keywords: Desvenlafaxine Succinate, Spectrofluorimeter, Precision, Accuracy, Reproducible

INTRODUCTION

Desvenlafaxine succinate (Fig: 1) is a newer antidepressant drug, which is chemically 1-[(1RS)-2-(dimethylamino)-1-(4-hydroxyphenyl) ethyl] cyclohexanol succinate monohydrate which is chemically unrelated to tricyclic, tetra cyclic, or other available antidepressant agents. Desvenlafaxine succinate is a structurally novel SNRI [1-2] (serotonin-norepinephrine reuptake inhibitor) and has no affinity for muscarinicccholinergic receptors i.e., it works by blocking the transporter "reuptake" proteins and there by increases neurotransmitters in brain synapses which drastically affects mood [1-2]. It is useful for the treatment of MDD (major depressive disorder), generalized anxiety and panic disorders.

Desvenlafaxine (O-desmethyl venlafaxine) is the major active metabolite of the antidepressant venlafaxine. Preclinical studies have shown that venlafaxine and its major metabolite, O-desmethylvenlafaxine (ODV), are potent inhibitors of serotonin and noradrenaline reuptake, and also weakly inhibit dopamine reuptake. Venlafaxine and its major metabolite appear to be equipotent with respect to their overall action on neurotransmitter re-uptake and receptor binding. Studies in animals show that tricyclic antidepressants may reduce adrenergic receptor responsiveness following chronic administration. In contrast, venlafaxine and ODV reduce adrenergic responsiveness after both acute (single dose) and chronic administration.ODV exists in enantiomeric forms in which R-enantiomer being more potent.

Desvenlafaxine succinate is not official in any pharmacopoeia. Literature survey revealed that HPLC methods coupled with tandem mass spectrometry [3-6], Adsorptive stripping differential pulse voltammetric method [7], bar sorptive extraction methods [8] and UV spectrophotometric methods [9], were reported for the estimation of desvenlafaxine. But there was no spectrofluorimetric method reported for the estimation of desvenlafaxine succinate in bulk form and in tablet dosage form. This paper describes simple and sensitive spectrofluorimetric method for the determination of desvenlafaxine succinate in bulk and in tablet dosage form.
Spectrofluorimetric method has advantages like high sensitivity, selectivity, easy to operate, economic and could be easily adapted for routine quality control analysis, dissolution or similar

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studies. In this method Desvenlafaxine Succinate showed excitation wavelength at 274 nm and emission wavelength at 305nm. The developed method was validated according to ICH Guidelines [11] and also used to estimate the total drug content in the commercial formulation of Desvenlafaxine Succinate-ER tablets [10]. The results of the analysis were validated by statistical methods [12-13].

MATERIALS AND METHODS

Apparatus: Fluorescence spectra and intensity measurements were made on a Shimadzu RF-5301 PC spectrofluorimeter equipped with single quartz cell of 1 cm path length. METTLER-TOLEDO AG-135 analytical electronic balance was used for weighing the sample. pH tutor (EUTECH Instruments), Ph meter was used to measure the pH of solutions.

Reagents: Pure Desvenlafaxine Succinate was provided by Torronto Research Centre (Canada). All other reagents were of Analytical Grade purchased from S.D. Fine Chemicals Ltd., Mumbai, India. The commercially available marketed tablet, Ventab Dxt 50 (Intas Pharmaceuticals Ltd., Ahmadabad) containing 50 mg of desvenlafaxine Succinate was used and it was procured from the local market. All solutions were prepared in triply distilled water. The materials and vessels used for trace analysis were precleaned by soaking in sulfochromic acid mixture (saturated $K_2Cr_2O_7$in concentrated H_2SO_4) for at least 1 hr and subsequently rinsed with triply distilled water before use.

METHOD DEVELOPMENT

Solvent Selection: To develop a rugged and suitable spectrofluorimetric method for the analysis of Desvenlafaxine Succinate in pure form and in formulation, different solvents systems ranging from polar to non polar were used. The drug was found to be freely soluble in methanol and dimethyl formamide, soluble in water, 0.1 M hydrochloric acid, 0.1 M sodium hydroxide, and phosphate buffer pH 4, 6, 6.8 slightly soluble in chloroform, sparingly soluble in ethanol, very slightly soluble in n-hexane and n-butane. Combination of buffers pH 6, 6.8 with different concentrations of acetonitrile and methanol were also investigated. The criteria employed for assessing the suitability of a particular solvent system for the drug was cost, stability, sensitivity of the assay and use of the same solvent system for extraction of the drug from the formulation excipient matrix for estimation of the drug content. Finally phosphate buffer pH =6 was chosen as a solvent system as it fits the above mentioned criteria.

Preparation of Phosphate Buffer pH 6: To 50.0 ml of 0.2 M potassiumdihydrogen phosphate in a 200-ml volumetric flask, 5.6ml of 0.2 M sodium hydroxide was added and then water was added to make upto 200ml.(According to Indian Pharmacopoeia-2007)

Preparation of standard stock solution: A 100 µg/ml primary standard stock solution of Desvenlafaxine succinate was prepared by dissolving 10.0 mg of drug in little quantity of buffer and diluting it to the mark in a 100 ml volumetric flask with the same buffer.

Selection of excitation and emission λ_{max}: The secondary standard stock solution (10µg/ml) was scanned between 200 and 400 nm for excitation wavelength keeping 300nm as emission wavelength. From the spectrum obtained, 274 nm was selected as excitation λ_{max} for the analysis of desvenlafaxine succinate .Then keeping the excitation wave length as 274 nm the stock solution was scanned between 300nm-500nm for emission wavelength using same solvent system as blank. From the spectrum obtained, 305 nm was selected as emission λ_{max} for the analysis of desvenlafaxine succinate.

Calibration graph: From the primary standard stock solution, various dilutions were made to obtain solutions of 100, 300, 500, 700, 900 ng/ml with the same solvent system just before use and fluorescence intensity was measured for each dilution at 274nm as excitation wavelength and 305 nm as emission wavelength. Five sets of Calibration graph standards were analysed to avoid variation. The calibration curve was plotted between concentration and average Fluorescence intensity in the concentration range from 100 to 900ng/mL. The results were listed in Table 1.

METHOD VALIDATION

Linearity: Five separate series of working standards of the drug ranging from 100 – 900ng/ml were prepared from the stock solution and analyzed. Linearity plot was plotted between fluorescence intensity/conc. vs concentration of respective working standards.

Accuracy: The accuracy of the method was checked by recovery determinations. The determination was done over three concentration levels in triplicate according to the ICH guidelines. The concentration levels selected as Quantifiable

Concentrations were 200ng/ml, 500ng/ml and 800ng/ml.

Precision: Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability refers to the use of the analytical procedure within a laboratory over short period of time that was evaluated by assaying the Quantifiable Concentration (QC) samples during the same day and on different times using new solutions. Intermediate precision was assessed by comparing the assay on different days (3 days), three replicates of each QC sample pool at low (200ng/ml), middle (500ng/ml), and high (800ng/ml) concentrations were assayed according to the ICH requirements [15]. Percentage relative standard deviation (%RSD) was calculated.

Selectivity / Specificity: During specificity study different solutions were prepared each having desvenlafaxine Succinate and other ingredients used in tablet preparation. The spectra and purity plots were traced for each ingredient in the standard.

Robustness and Ruggedness: The robustness was determined by preparing stock solution in pH =6.2 phosphate buffer system, while the Ruggedness of the method was established by changing the analyst and the percent recovery was determined. The results obtained were subjected to unpaired t-test with actual percent recovery values to find any significant difference.

Limit of detection (LOD) and quantification (LOQ): LOD and LOQ were calculated based on Standard Deviation of response and Slope of calibration curve. LOD and LOQ were calculated from the formula 3.3σ/S and 10 σ/S respectively where 'σ' is Standard deviation of the y-intercept of the regression line and 's' is slope of the calibration curve.

ESTIMATION OF DESVENLAFAXINE SUCCINATE FROM COMMERCIAL FORMULATION BY THE PROPOSED METHOD: Ten tablets of formulation (Ventab Dxt 50) containing 50 mg of Desvenlafaxine Succinate were accurately weighed and crushed. From the powdered tablets weight equivalent to 2.5 mg of desvenlafaxine succinate was taken and extracted with 100ml pH =6 phosphate buffer by sonicating for 30 minutes followed by filtration through Whatman filter paper No. 41. From this solution, suitable dilutions were made to fit the concentration into calibration curve range.

Recovery Studies: The accuracy of the proposed method was further established by using recovery studies, i.e., by external dilution method. The known amount of standard pure drug was added at 3 different levels i.e. 80%, 100 % and 120 % to weight of powdered tablet equivalent to 2.5 mg of Desvenlafaxine Succinate. Each Solution was suitably diluted so that Fluorescence intensity lies within the calibration curve range and then the percent recoveries were calculated. The determinations were done in triplicate according to the ICH guidelines.

RESULTS AND DISCUSSION

Though different solvent systems were tried, phosphate buffer pH =6 was selected as it was economical, none polluting and drug sensitivity as well as stability were found to be high. Excitation and Emission wavelengths were found to be 274 and 305 nm respectively in the selected solvent system. Calibration Curve was plotted within the concentration range of 100-900ng/ml as shown in Fig: 2. and it was found to be linear with the regression coefficient of 0.999 (Table 2) which was highly significant for the method developed. The linear regression equation obtained was Y = 0.396X - 1.701, where Y is the Fluorescence intensity and *X* is the concentration (in ng/ml) of pure Desvenlafaxine Succinate solution. The statistical analysis of data obtained for the estimation of Desvenlafaxine Succinate in pure solution indicated a high level of precision for the proposed method as evidenced by low standard deviation values and low values of coefficient of variation (Table 1). A one-way ANOVA test [12-13] (Table 3) which was performed on the values observed for each pure drug concentration during the replicate measurement of the Calibration Curve standards indicates that there was a less variation among the replicates as calculated *F*-value (F_{Calc}) was found to be less than the critical *F*-value (F_{Crit}) at 95% significance level. In Selectivity studies excepients used in tablet preparation didn't show any interference in the spectrum of drug which indicates that method is highly selective for the Desvenlafaxine Succinate. Hence the developed method can be employed for the estimation of Desvenlafaxine Succinate in the commercial preparations

The developed method was validated according to the ICH Guidelines and the results obtained were tabulated in Table 4 and 5. The linearity range was found to be 100 – 900 ng/ml as shown in Fig: 3. the accuracy of these estimations was calculated on the basis of percent recovery and results varied between 99.82–100.78% for the QC standards selected which indicates that the method was highly accurate. Repeatability and intermediate precisions calculated on the basis of percentage relative standard deviation on replicate set of

selected QC standards (*n* = 3 for each concentration) were found to be less than 2%(Table 4) which indicates that developed method can produce reproducible results at different times and also on different days. For the developed method, varying the pH of phosphate buffer from 6 to 6.2 did not significantly affect the percent recovery at different QC standards, which was inferred from unpaired t-test at 95% confidence level indicating that method was robust at slight variations of experimental procedures. Similarly no significant difference in the percent recoveries at different QC standards by varying analysts at 95% confidence interval by applying unpaired T-test was observed indicating that the method was rugged due to change in analysts or experimental conditions. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 6.14 ng/ml and 18.61 ng/ml as given in Table 5 which indicates that the method is highly sensitive.

The method was evaluated by estimating the amount of Desvenlafaxine Succinate in commercially available formulation (Ventab Dxt 50) by the proposed method and analysis of pure drug solution as reference. The results are presented in Table 6. The percentage recovery from this formulation by the proposed method was 98.36% with a coefficient of variation of 1.92. Whereas for the pure drug the percent recovery was found to be 99.69% with a coefficient of variation of 0.32. In both the cases percent recovery was more than 98 % which indicates that the proposed method can be used for routine analysis of Desvenlafaxine succinate in pharmaceutical formulations. The accuracy of the method was further established by external dilution recovery studies at three different levels i.e. at 80%, 100% and 120%.The results were tabulated in Table 7. Percent Recovery was found to be varying between 97.54 to 98.94% indicating that the method was highly accurate and reliable.

CONCLUSION

The method was validated with respect to linearity, precision, accuracy, selectivity, Robustness and Ruggedness. The calibration plot and linearity plot for the method was constructed. The regression equation and correlation coefficient of the mean of five consecutive calibrations were given. Accuracy was investigated by analyzing marketed formulations and percentage was found to be in the range of 98-101 %. Low relative standard deviation (RSD) values of Repeatability and interday precision indicates that the method was highly reproducible. By applying the standard addition technique further assessed the validity of the suggested method. It was carried out by adding a known amount of sample to the marketed formulation at 80-120 % of concentration according to ICH guidelines .The percentage recoveries of the concentration were found to be more than 95%. When compared to previously reported liquid Chromatographic method this method is rapid and do not require any expensive or sophisticated instruments and chemicals and when compared to reported UV Spectrophotometer method this method is highly sensitive i.e. by this method even nanogram level concentration can also be detected. Thus the proposed method based on spectrofluorimeter is precise, accurate, simple and economic to perform and hence it can be used for routine analysis for bulk and solid dosage forms.

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Table 1: Calibration curve points of the proposed method for estimation of Desvenlafaxine Succinate

Concentration of the solution (ng/ml)	Mean absorbance value*	Coefficient of variation (%)
100	39.20 ± 0.37	0.94
300	116.36 ± 0.40	0.35
500	194.59 ± 0.30	0.15
700	276.42 ± 3.77	1.36
900	355.34 ± 2.65	0.74

*Average of five determinations with standard deviation.

Table 2: Results of least square regression analysis of data for the estimation of Deslvenlafaxine Succinate by the proposed method

Statistical parameters	Value[a]
Regression equation	Y = 0.396X - 1.701
Correlation coefficient (r^2)	0.999
Standard error of slope	3.75×10^{-2}
Standard error of intercept on ordinate	0.7370

[a]Based on five calibration Curve values.

Table 3: One-way ANOVA test to check variation in five sets of Calibration Curve Values

Source of Variation	Degree of freedom (DF)	Sum of squares (SS)	Mean sum of squares (MS)	*F* -value	
				F_{calc}	F_{crit}*
Between Sets	4	39.44	9.860	0.00062	2.87
Within Sets	20	314000	15700		
Total	24	314000			

* Theoretical value of *F* (4, 20) based on one-way ANOVA test at *P*=0.05 level of significance.

Table 4: Results of Validation parameters

Analytical parameter	200 ng/ml	500ng/ml	800ng/ml
Accuracy (%RECOVERY)	100.52	100.78	99.82
Repeatability (%RSD)	1.08	0.97	0.66
Intermediate precision (%RSD)	1.99	1.55	0.72
Ruggedness (t_{cal})	0.24	0.07	0.38
Robustness (t_{cal})	0.44	0.30	0.34

t_{tab} value at 95% confidence interval, d.f = 4, two-sided for robustness and ruggedness is 2.78

Table 5: Results of other Validation parameters

Analytical parameter	Value
Linearity	100-900ng/ml
Limit Of Detection (LOD)	6.14ng/Ml
Limit of Quantification (LOQ)	18.61ng/ml

Table 6: Results of the assay of pure desvenlafaxine succinate and commercial formulations by the proposed method

Sample	Label claim (mg/capsule)	Results	
		AR (%)	CV%[a]
Pure drug solution[b]		99.69	0.32
Commercial Form	100	98.36	1.92

CV, coefficient of variation; AR, mean analytical recovery.
a CV for triplicate determinations.
b 10 mg in 100 ml.

Table 7: Results of the Recovery studies

S.No.	Percent Pure Drug Added	AR (%)	CV%[a]
1	80	98.94	1.56
2	100	98.48	1.75
3	120	98.69	1.98

CV, coefficient of variation; AR, mean analytical recovery.
a CV for triplicate determinations.
Note: In each determination powdered drug equivalent to 2.5mg Was taken and to it respective amount of pure drug was added.

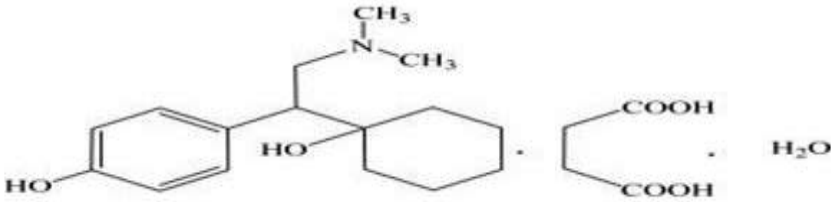

Fig.1. Structural formula of Desvenlafaxine Succinate

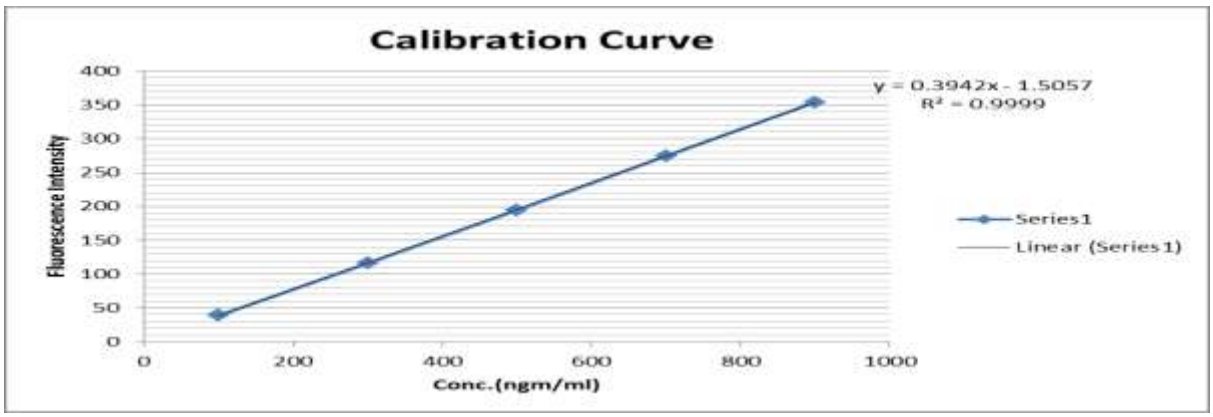

Fig 2: Calibration Curve plotted based on mean fluorescence intensity of five sets Vs conc. (ng/ml)

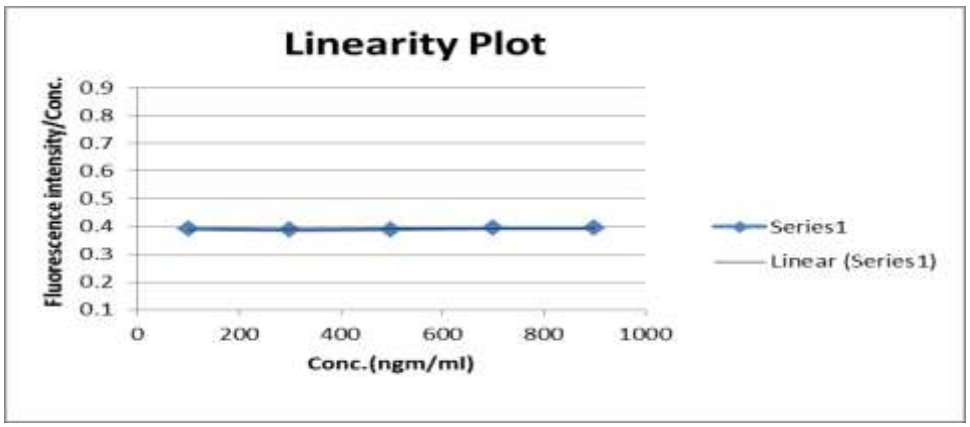

Fig 3: Linearity Plot Plotted based on mean fluorescence intensity of five sets/Conc.

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