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Validated Analytical method Development of Desvenlafaxine succinate in solid dosage form by RP-HPLC and HPTLC methods

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ABSTRACT

The objective of this work was to develop and validate simple, rapid and accurate chromatographic methods (A and B) for determination of Desvenlafaxine succinate in solid dosage form. In method A - RP-HPLC method was based on Reversed Phase High Performance Liquid Chromatography, on ODS C_{18} RP column (150 mm × 4.6 mm i.d., 5 µ), using Methanol : 50 mM Phosphate buffer (pH 8.0): Acetonitrile (50:40:10 % v/v) as the mobile phase, at a flow rate of 1 mL/min at ambient temperature. Quantification was achieved by UV detection at 225 nm over a concentration range of 5-25 µg/mL for Desvenlafaxine succinate. The mean retention time for Desvenlafaxine succinate was found to be 4.80 min. The amount of Desvenlafaxine succinate estimated as percentage label claim was found to be 99.83 ± 1.1093. In method B - HPTLC method was based on TLC separation of the drug using silica gel 60 F 254 aluminium sheets and Chloroform : Methanol : Water (60:30:10 v/v/v) as mobile phase. Detection was carried out at 226 nm over the concentration of 1 - 3.5 µg/mL Desvenlafaxine Succinate. The mean R_f value of Desvenlafaxine succinate was found to be 0.63. The amount of Desvenlafaxine succinate was estimated as percentage label claim found to be 101.57 ± 0.92668. Both of these methods were found to be simple, precise, accurate, selective and could be successfully applied for determination of pure laboratory prepared mixture and tablet.

Key words: RP-HPLC, HPTLC, Desvenlafaxine succinate, marketed formulation.

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INTRODUCTION

Desvenlafaxine succinate is a newer antidepressant drug, which is chemically 1-[(1RS)-2-(Dimethylamino)-1-(4-hydroxyphenyl) ethyl] cyclohexanol succinate monohydrate. Desvenlafaxine succinate is a structurally novel SNRI (serotonin - norepinephrine reuptake inhibitor) useful for the treatment of MDD (major depressive disorder). Desvenlafaxine (O-desmethyl venlafaxine) is the major active metabolite of the antidepressant venlafaxine, a medication used to treat major depressive, generalized anxiety and panic disorders. Desvenlafaxine succinate is not official in any pharmacopoeia. Literature survey revealed that HPLC and LC-MS[1-4] methods were reported for the estimation of Desvenlafaxine Succinate in human plasma and LC method[5] are reported for the estimation of Desvenlafaxine Succinate in human plasma and whole blood. In the present investigation, we report the HPLC and HPTLC Methods for Determination of Desvenlafaxine Succinate in Tablet Dosage form.

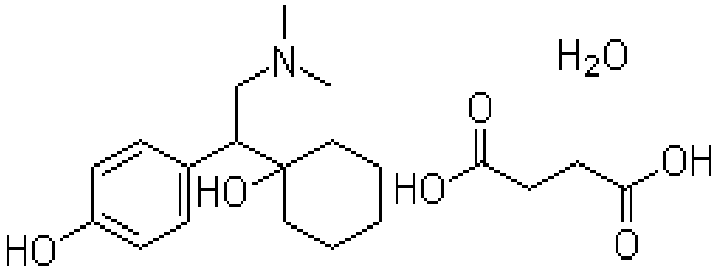


Figure. 1 Stucture of Desvenlafaxine Succinate

MATERIALS AND METHODS

Materials

Pharmaceutical grade of Desvenlafaxine succinate was kindly gifted from Orchid Pharma, Chennai. The commercially available marketed tablet Ventab Dxt 50 (Intas Pharmaceuticals Ltd., Ahmadabad) containing 50 mg Desvenlafaxine was used and it was procured from the local market. All the solvents and chemicals used were Water, Methanol, Potassium dihydrogenortho phosphate, Triethylamine, Acetonitrile are of HPLC grade were used in the present investigation from Qualigens fine chemicals, Mumbai.

Instruments

Shimadzu AUX-220 balance, Microprocessor based pH tester from Eutech and Qakton instruments, SPD – 10 Avp / 10 Avp Shimadzu UV- Visible detector, LC - 10 ATvp Shimadzu solvent deliver module, a ODS C_{18} RP column (150 mm × 4.5 mm i.d., 5µ), 50 µL ASGE glass syringe, Ultra sonicator mod 2200 MH, CAMAG Automatic TLC Sampler 4 (ATS4)" and CAMAG TLC scanner 3"scanner 3_140703" were used.

Method A - RP-HPLC Method

Preparation of Mobile Phase

The 50 mM phosphate buffer was prepared[6] and pH adjusted to 8 and filtered through cellulose acetate filter paper using vacuum filtration and mixed with methanol and acetonitrile. A mixture of Methanol, 50 mM Phosphate buffer and Acetonitrile in the volume ratio of 50:40:10 v/v/v was sonicated for 15 minutes to degas the mobile phase.

Preparation of standard stock solution

An accurately weighed quantity of 25 mg of Desvenlafaxine succinate was dissolved in a minimum quantity of methanol, the total volume was made up to 10 mL with more amount of methanol (2.5 mg/mL). Further dilution was made by diluting 1 mL to 50 mL with methanol to obtain 50 µg/mL solution.

Linearity and calibration graph

In this method, the aliquots of stock solution of Desvenlafaxine succinate (1-5 mL of 50 µg/mL) were transferred into five 10 mL volumetric flasks and made up to the mark with mobile phase. A solution contains 5, 10, 15, 20 and 25 µg/mL of Desvenlafaxine succinate in mobile phase. The solutions (20 µL) were injected and the chromatograms were recorded at 225 nm. It was found that the above concentration range was linear with the concentration range of 5-25 µg/mL. The procedure was repeated for three times. The peak area was plotted against concentration and the calibration curve was constructed.

Quantification of formulation

Twenty tablets of marketed formulation (Ventab Dxt 50) containing 50 mg of Desvenlafaxine were weighed accurately and the average weight was found and powdered. The tablet powder equivalent to 25 mg of Desvenlafaxine succinate was weighed and added a minimum quantity of methanol to dissolve substance, the total volume was brought to 10 mL with methanol (2.5 mg/mL), and the solutions were sonicated for 10 minutes and filtered through Whatmann filter paper No.41. From the clear solution, further dilution was made by diluting 1 mL into 50 mL with mobile phase to obtain 50 µg/mL solution. 2 mL of solution (50 µg/mL) was taken into 10 mL volumetric flask and made up to mark with mobile phase. With optimized chromatographic conditions, a steady baseline was recorded for about 75 minutes. After the stabilization, solution (20 µL) was injected after filtering through 0.2 µ membrane filter and recorded the chromatogram. The peak area of the eluted chromatograms were observed and the amount was calculated by using average of slope and intercept values.

Recovery studies

The recovery analysis was performed by adding known concentration of Desvenlafaxine succinate working standard to the pre-analyzed formulation. To the pre-analyzed tablet powder equivalent to 25 mg (labeled claim of tablet), known quantities of standard drug (80,100 and 120 % of quantification concentration) were added separately and contents were made up to the mark. The solutions were sonicated for 15 minutes. After sonication the solutions were filtered through Whatmann filter paper No.41. The solutions were injected and the chromatograms were recorded at 225 nm. The amount of drug recovered from the formulation was calculated by using average of slope and intercept values. The procedure was repeated for three times at each level.

Validation[7]

Precision

To study reproducibility of the method, precision was carried. Exercise was initiated with running six replicates of test solution of analyte. Mean response was calculated followed by calculation of relative standard deviation. For determination of method precision, six different solutions of sample were prepared and injected into the chromatographic system.

Accuracy

To study the closeness of the results, accuracy was carried. The three replicate recovery studies were performed by adding increasing concentrations of raw material solution (standard addition method) to a fixed concentration of formulation solution, mixed well, volume made and reanalyzed.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Preparation of calibration curve from the several dilutions of standard was repeated for three times. The LOD and LOQ were calculated by using the average values of slope and standard deviation of intercept values and were found to be 0.038192475 µg/mL and 0.115734773 µg/mL.

System Suitability Studies[8]

Weighed accurately 25 mg of Desvenlafaxine Succinate in 50 mL methanol, transferred 1 mL of the solution into a 50 mL standard flask and made up the volume with methanol and pipetted out 2 mL of the solution into a 10 mL standard flask and volume was made with the mobile phase and the solution was injected under optimum chromatographic conditions. The chromatogram obtained was tested for its acceptance using parameters like column efficiency, tailing factor, asymmetric factor and capacity factor.

Method B - HPTLC Method

Preparation of Mobile Phase

The mobile phase was prepared by using Chloroform, Methanol, and Water in the volume of ratio 60:30:10 v/v/v.

Preparation of standard stock solution

A standard stock solution of Desvenlafaxine succinate was prepared by dissolving in methanol to produce 100µg/mL and the solution was used to establish linearity.

Evaluation of linearity

From stock solution of 100 µg/mL, From this 1-3.5 mL were pipetted out into a series of six 10 mL volumetric flasks, a final concentration of Desvenlafaxine succinate ranging from 1 to 3.5 µg/mL was obtained and this solution was spotted on a pre-coated TLC plates and developed as per the procedure discussed the peak area obtained for the different concentration.

Calibration graph

A graph of peak area against concentration was constructed for Desvenlafaxine succinate in the concentration range of 1 to 3.5µg/mL and it was found to be linear.

Analysis of sample

Twenty tablets of marketed formulation (Ventab Dxt 50) containing 50 mg of Desvenlafaxine were accurately weighed and the average weight was found and powdered. The powdered tablet equivalent to 10 mg of Desvenlafaxine succinate was transferred into a 100 mL volumetric flask, added 25 mL of methanol and sonicated for 15 min, then shaken vigorously for few min and finally made up to the mark with methanol. The above solution was collected by filtering it through Whatmann filter paper No.41. From the filtered solution, 2.5 µg/mL was spotted on a TLC aluminum sheets silica gel 60 F 254 plates and the plates are allowed to develop in Twin Trough Chamber 20 × 10 cm using Chloroform: Methanol: water, the solvent front position is noted, the plates are then removed and allowed it to dry in oven at 60˚c for 5 min.

The spots are then detected using Camag TLC scanner 3 and the peak area obtained at the detecting wave length 226 nm, the amount of Desvenlafaxine succinate was calculated using the regression equation.

Recovery studies

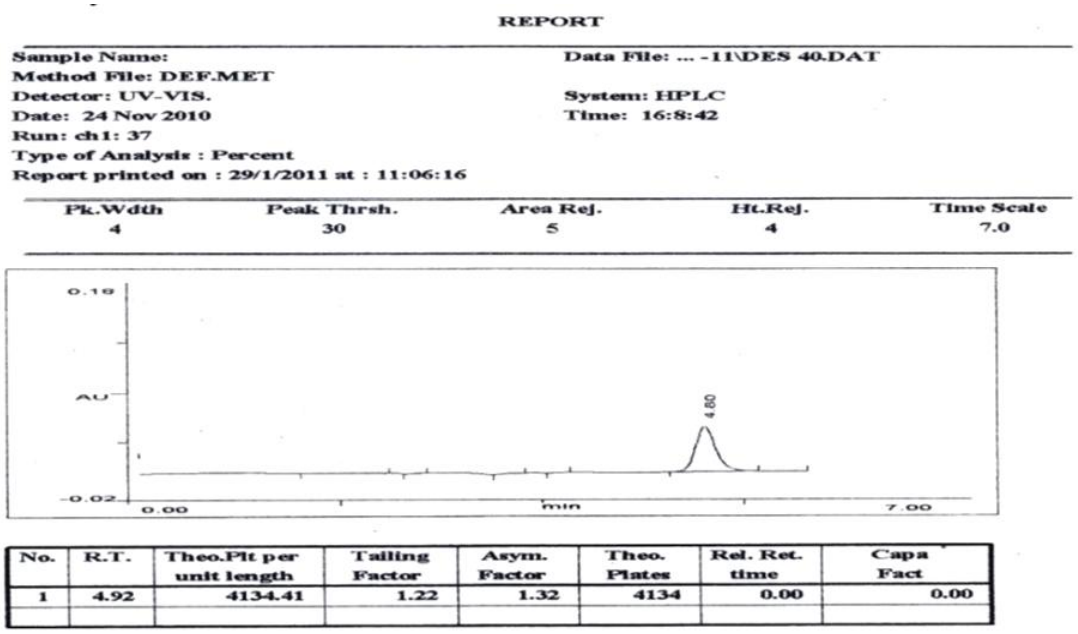
The recovery studies were carried out by adding a known quantity of the standard drug to the pre-analyzed formulation and the whole contents was re-analyzed by the proposed method. The percentage recovery was calculated and the data was tabulated.

Statistical Validation[9]

The obtained results were treated for statistical validation parameters like Standard Deviation (SD), Percentage Relative Standard Deviation (% RSD) and Standard Error.

RESULTS AND DISCUSSION

An effort has been made to identify simple, cost effective, economic, specific and accurate methods for the estimation of Desvenlafaxine succinate in pure form and in formulation.



REPORT

Sample Name: Data File: ... -11\DES 40.DAT
Method File: DEF.MET
Detector: UV-VIS. System: HPLC
Date: 24 Nov 2010 Time: 16:8:42
Run: ch1: 37
Type of Analysis : Percent
Report printed on : 29/1/2011 at : 11:06:16

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	7.0

No.	R.T.	Theo.Plt per unit length	Tailing Factor	Asym. Factor	Theo. Plates	Rel. Ret. time	Capa Fact
1	4.92	4134.41	1.22	1.32	4134	0.00	0.00

Figure. 2: HPLC Chromatogram of Desvenlafaxine succinate

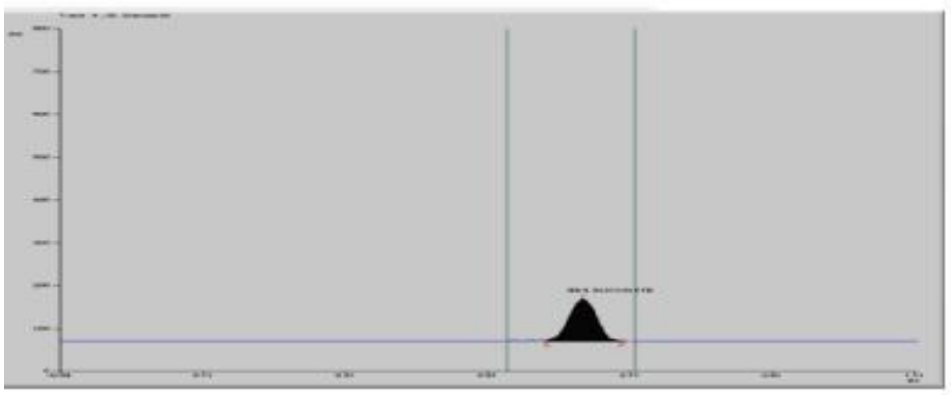


Figure.3: HPTLC Chromatogram of Desvenlafaxine succinate

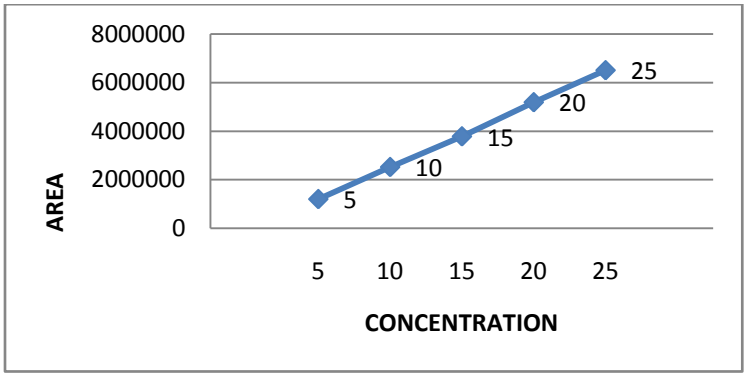


Figure. 4: Calibration Graph OFHPLC

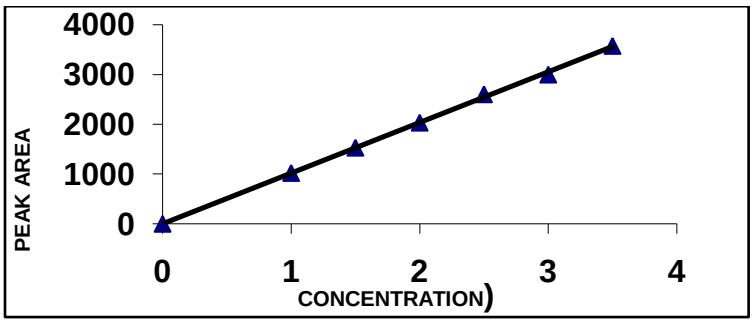


Figure. 5 : Calibration Graph Of HPTLC

Table.1 Optical Characteristics Of Desvenlafaxine succinate

S.No	Parameters	Method A	Method B
1.	λmax (nm)	225	226
2.	Sand ell's sensitivity (μg/cm^2/0.001A.U.)	3.82138 × 10^{-09}	9.90831 × 10^{-07}
3.	Correlation coefficient (r)	0.9996	0.9992
4.	Régression équation (y = mx + c)	Y =261686.16x-64028.5	Y=1009.25x +9.9385
5.	Slope (m)	261686.16	1009.25
6.	Intercept (c)	0-64028.5	9.9385
7.	Standard error of mean	5913.845536	3.17764 × 10^{-15}

Table.2: Analysis of Commercial Formulation

Drug	Method	Sample No	Labeled Amount (mg/tab)	Amount found (mg/tab)	Percentage obtained (%)	Average	S.D.	%R.S.D.	S.E.
Ventab Dxt 50	Method A	1	50	50.05	100.11				
		2	50	49.90	99.80				
		3	50	49.95	99.10	99.83 %	1.1093	1.1111	0.03081
		4	50	50.56	101.12				
		5	50	50.38	100.77				
		6	50	50.55	100.10				
	Method B	1	50	51.26	101.52				
		2	50	50.46	100.92				
		3	50	50.42	100.85	101.57 %	0.9266	0.9123	0.02574
		4	50	50.08	100.17				
		5	50	50.24	100.48				
		6	50	50.26	100.52				

In the RP-HPLC method, Methanol: 0.05 M phosphate buffer pH 8.0: Acetonitrile (50:40:10v/v/v) was selected as mobile phase and showed λmax at 225 nm. This is shown in Figure.1, with linearity of 5 – 25 μg/mL. In HPTLC method, Chloroform: Methanol: Water (60:30:10v/ v/v) was selected as mobile phase. After eluting, the spots were scanned in the TLC chamber. From that, 226 nm was selected as analyzing wavelength and shown in Fig.2, with linearity range of 1 – 3.5 μg/mL. Beer's Law limits, Molar absorptivity, Sand ell's sensitivity[10], Slope and Intercept for the methods A and B are shown in the Table 1.

The assay for brand of Desvenlafaxine Succinate by RP-HPLC method for Ventab Dxt 50 was found to be 99.83 ± 1.1093 and by HPTLC method for Ventab Dxt 50 was found to be 101.57 ± 0.92668 are shown in Table 2.

The recovery studies results by RP-HPLC method for Ventab Dxt 50 was found to be 100.17 % and by HPTLC method for Ventab Dxt 50 was found to be 98.97 % are shown in Table 3. The values of co-efficient of variance were satisfactorily low and recovery was close to 100 % indicating reproducibility of the methods. The excipients in the formulation did not interfere in the accurate estimation of Desvenlafaxine Succinate in tablet dosage form.

From the results, both the methods are simple, linear, rapid, precise and economic. Hence it can be effectively applied for the routine quality control purpose of Desvenlafaxine Succinate in bulk and in tablet dosage forms.

Table.3: Recovery studies

Method	Recovery(%)	Average	S.D.	% R.S.D.	S.E.
Method A	101.37 100.50 98.66	100.17 %	1.38363	1.38119	0.038434
Method B	99.33 98.00 99.60	98.97 %	0.856524	0.86538	0.023792

Table.4: System Suitability parameters for RP-HPLC Method

S.No	Parameters	Values
1.	Retention time	4.80 min
2.	Flow rate	1 mL/min
3.	Tailing factor	1.38
4.	Asymmetry factor	1.65
5.	LOD (µg/mL)	0.038192475
6.	LOQ (µg/mL)	0.115734773

CONCLUSION

The standard deviation, %RSD and standard error calculated for both the method are low, indicating high degree of precision of the method. The % RSD is also less than 2% as required by ICH guidelines. Hence the developed methods are simple, rapid, precise, accurate, and cost effective and can be employed for the routine analysis of Desvenlafaxine Succinate in both bulk and in tablet dosage form.

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