

## VALIDATED HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY METHOD FOR DETERMINATION OF DESVENLAFAXINE SUCCINATE IN TABLET

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### ABSTRACT

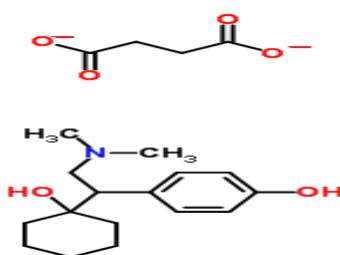
A simple validated high-performance thin-layer chromatographic method has been proposed for the determination of Desvenlafaxine succinate in a tablet dosage form. The separation was achieved on silica gel 60 F<sub>254</sub> coated aluminum sheet as stationary phase using ethyl acetate: n-hexane: triethyl amine (5.0: 4.5: 0.5 v/v/v) as mobile phase which gave compact spots with R<sub>f</sub> value 0.60 ± 0.02. Quantitative densitometric evaluation was done in absorbance-reflectance mode at 226 nm. The response was found to be linear over concentration range of 100-500 ng/spot with correlation coefficients 0.991 and 0.999 and mean percentage recovery of the drug was observed to be 99.33 ± 0.561 and 99.28 ± 0.287 by peak area and peak height, respectively. The method was validated for linearity, accuracy, range, precision and robustness according to ICH Q2 (R1) guidelines. The method is simple, accurate, precise and was successfully applied to the assay of drug in tablet formulation.

**KEYWORDS:** Desvenlafaxine succinate, Assay, HPTLC, Validation.

### INTRODUCTION

Desvenlafaxine succinate (DVS) is a selective nor-epinephrine reuptake inhibitor and is used primarily for the treatment of major depression in adults. Chemically, it is 4-[2dimethylamino-1-(1-hydroxycyclohexyl) ethyl] phenol. It is also known as O-desmethylvenlafaxine and is a major active metabolite of Venlafaxine which presents an

activity profile similar to that of venlafaxine.<sup>[1]</sup> DVS, the succinate salt of the isolated major active metabolite of venlafaxine is a pharmacologically distinct molecule currently in clinical use for the treatment of major depressive disorder (MDD) and vasomotor symptoms (VMS) associated with menopause.<sup>[2],[3]</sup> A literature survey revealed analytical methods like HPLC-electro-spray ionization mass spectrometry (ESI/MS) for simultaneous determination of venlafaxine and its three metabolites in human plasma<sup>[4]</sup>, LC-UV and LC-MS methods have been developed and studied for stress conditions of Desvenlafaxine.<sup>[5]</sup> Stability indicating HPTLC method has been reported for its estimation.<sup>[6]</sup> An alternative HPTLC method for estimation of DVS in tablet formulation is described in the present article.



**Fig. 1: Chemical Structure of Desvenlafaxine succinate.**

## MATERIALS AND METHODS

### Reagents and Chemicals

Methanol, ethyl acetate, n-hexane, triethyl amine used were of AR grade, Merck India Ltd, Mumbai (India). Standard drug sample of Desvenlafaxine succinate (99.70% pure) was obtained as a gift sample from Wockhardt Pharmaceutical Limited, Aurangabad (India). The DVS tablets used in this study with a declared content equivalent to 50 mg Desvenlafaxine were procured from local market.

### Instrumentation and Chromatographic Conditions

#### Apparatus

HPTLC was performed with Camag HPTLC equipment comprising of Linomat IV sample applicator, Linomat Microliter syringe (*Hamilton- Bonaduz Schweiz*) 100  $\mu$ L, TLC Scanner-III with win CATS software version 1.4.1 for scanning and documentation, High-tech UV cabinet fitted with dual wavelength 254/ 366 nm, 8 volts UV lamps for visual inspection of HPTLC plates. 20 x 20 cm pre-coated Silica Gel 60 F<sub>254</sub> TLC aluminum plates (E. Merck, Darmstadt, Germany) with layer thickness 0.2 mm were cut to required size (10 x 10 cm) at the time of use. The TLC plates were washed with methanol by over-run technique and activated at 110 °C for 5 min. The samples were applied with Linomat IV Sample applicator

with the settings- band length, 4mm; distance between bands, 3 mm; distance from the plate side edge, 10 mm and distance from the bottom of the plate, 10 mm. Linear ascending development was performed in a 10 x 10 cm twin trough glass chamber with stainless steel lid, the chromatographic development was carried using ethyl acetate: n-hexane: triethyl amine (5.0: 4.5: 0.5 v/v/v) as mobile phase with chamber saturation time of 10 minutes, migration distance of 80 mm and application volume (5  $\mu$ L). After development, the plates were dried in a current of warm air and densitometric scanning was performed with a TLC Scanner III at 226 nm in absorbance- reflectance mode.

### Preparation of Desvenlafaxine succinate stock and working standard solutions.

#### a) Stock standard solution (Solution A)

An accurately weighed quantity of about 10.0 mg of DVS was dissolved in methanol and diluted to 10.0 mL (conc.: 1000.0  $\mu$ g/mL).

#### b) Working standard solution (Solution B)

A 0.6 mL of solution A was further diluted to 10.0 mL with methanol to obtain working standard solution (conc.: 60.0  $\mu$ g/mL).

After chromatographic development, bands were scanned over the range 200–400 nm and *in situ* spectrum were recorded and thus inferred that the estimations can be done at the maximum wavelength 226 nm. A representative chromatogram and *in situ* UV spectra are depicted in Fig. 1 & Fig. 2.

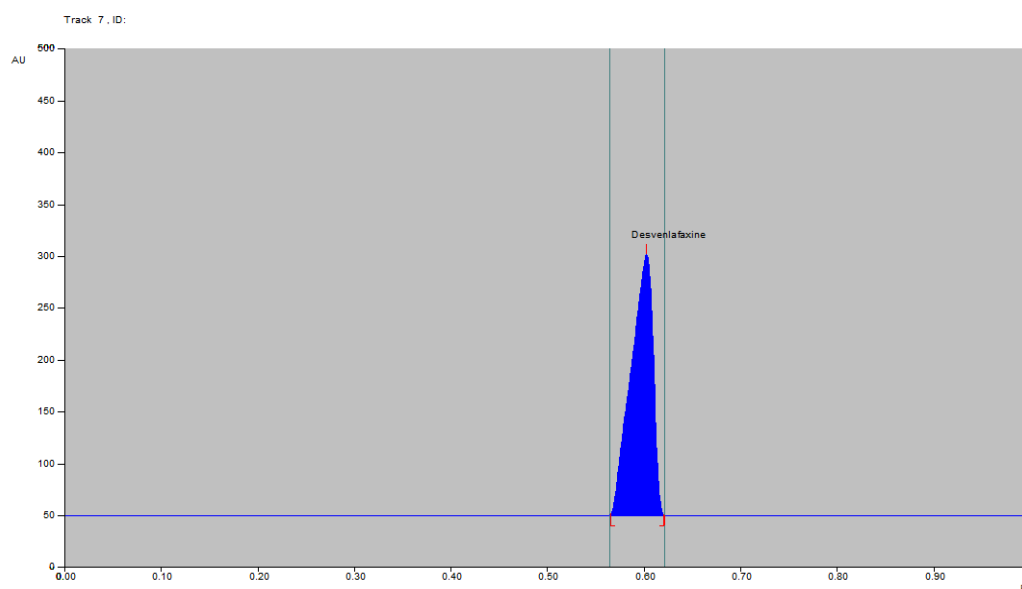
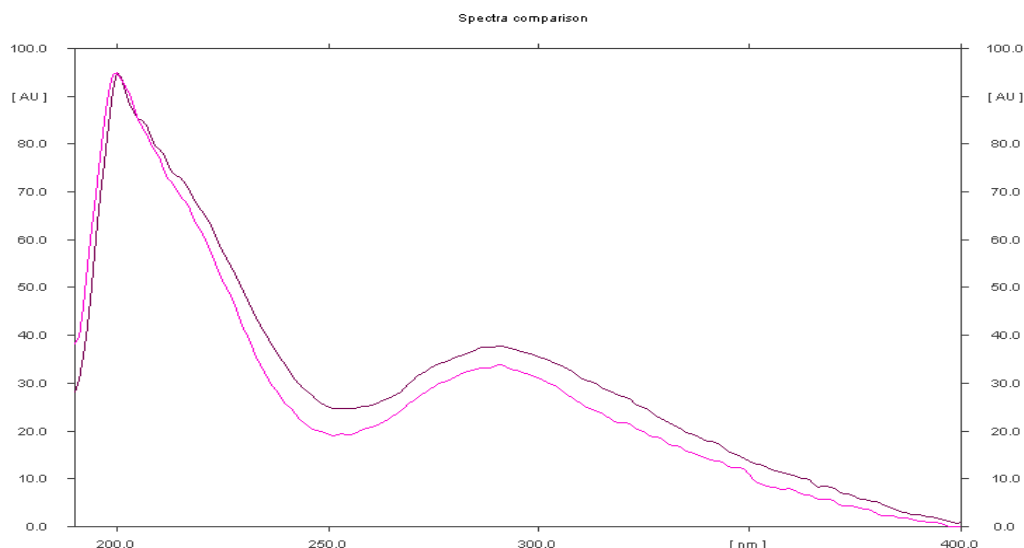


Fig. 2. Chromatogram of 100 ng/spot of DVS.



**Fig. 3. The overlain UV spectra of 100.0 µg/mL DVS (API and sample) between 200 and 400 nm.**

### ASSAY METHOD

Twenty tablets were weighed and crushed to a fine powder. An accurately weighed quantity of tablet powder equivalent to about 10.0 mg of DVS was taken into 10 mL volumetric flask, shaken with 8.0 mL methanol, sonicated for 5 minutes and the volume was made up to 10.0 mL with methanol and solution was filtered through Whatman Grade I filter paper. A 0.6 mL of the filtrate was diluted to 10.0 mL with methanol to get concentration of 60.0 µg/mL (on labeled claim basis).

**Procedure:** Two bands of working standard solution and six bands of sample solution of equal volume (5 µL) were applied on TLC plate, and the plate was developed and scanned as per optimized chromatographic conditions.

**Calculation:** Percent of labeled claim were calculated using following formula,

$$\% \text{ of Labeled claim} = \frac{\text{Au} \times \text{Wstd} \times \text{Wav}}{\text{Astd} \times \text{Ws} \times \text{Lc}} \times 100$$

where,

Au = area/height of sample peak

Astd = area/height of standard peak

Wstd = standard weight (mg)

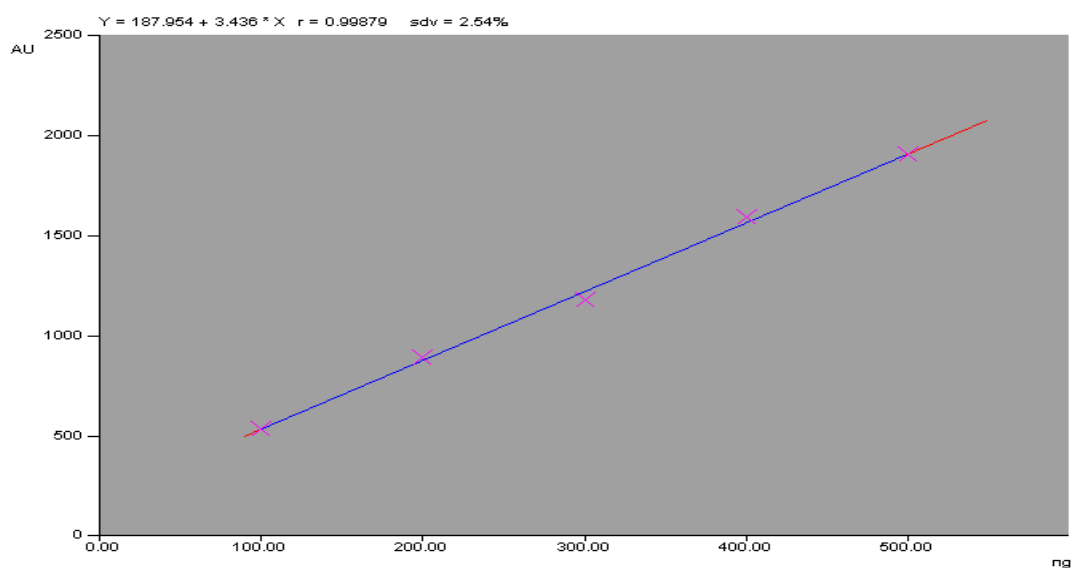
Ws = sample weight (mg)

Wav = average weight of tablet (mg)

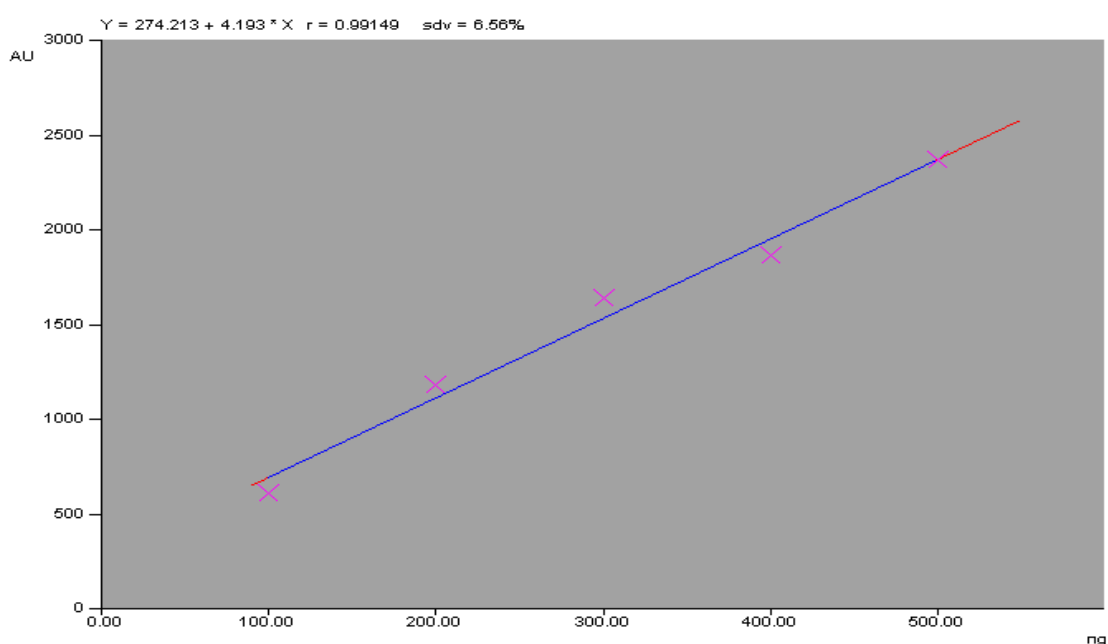
Lc = labeled claim (mg/tablet)

**METHOD VALIDATION<sup>[7]</sup>****LINEARITY OF RESPONSE**

Aliquot portions (5  $\mu\text{L}$ ) of series of standard solutions of five different concentrations of DVS 20.0, 40.0, 60.0, 80.0, 100.0  $\mu\text{g/mL}$  were applied in duplicate (100-500 ng/spot) on TLC plate and chromatograms were developed and scanned under optimized chromatographic conditions. The linear regression curves are depicted along with correlation coefficient; slope and y-intercept by peak height and area in Fig. 3 (a) & (b). The curves were found to be linear between concentration ranges 100-500 ng/spot.



(a)



(b)

**Fig. 4: Linearity Curve of DVS (a) Peak height (b) Peak area.**

**PRECISION****Repeatability**

Repeatability of results of assay by proposed method was ascertained by replicate analysis (n=6) of homogeneous sample of tablet powder. The results are shown in Table 1.

**Intermediate precision**

The samples were analysed by proposed method on different days (intra-day & inter-day), and by different analyst. The results of study are given in Table 1.

**ACCURACY (% RECOVERY)**

To check the accuracy of the method, recovery was measured by addition of standard drug at five different levels (70, 85, 100, 115 and 130% of labeled claim) to pre-analyzed sample. Accurately weighed quantities of pre-analyzed tablet powder equivalent to about 7.0 mg of DVS were transferred to five different 10.0 mL volumetric flasks and accurately weighed 1.5, 3.0, 4.5 and 6.0 mg of standard DVS were added to 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> flask respectively (representing 70-130% of labeled claim). This was followed by addition of about 8.0 mL of methanol in each flask and the contents were shaken and sonicated for 15 minutes. Sufficient methanol was added to each flask to adjust the volume to 10.0 mL and filtered. A 0.6 mL of each of the filtrate was diluted to 10.0 mL with methanol. Resultant sample solutions were analysed as described under assay method.

The percent recovery was then calculated at different levels of sample concentration using the formula:

$$\text{Recovery \%} = \frac{T}{B + C} \times 100$$

Where,

T = total drug estimated (mg)

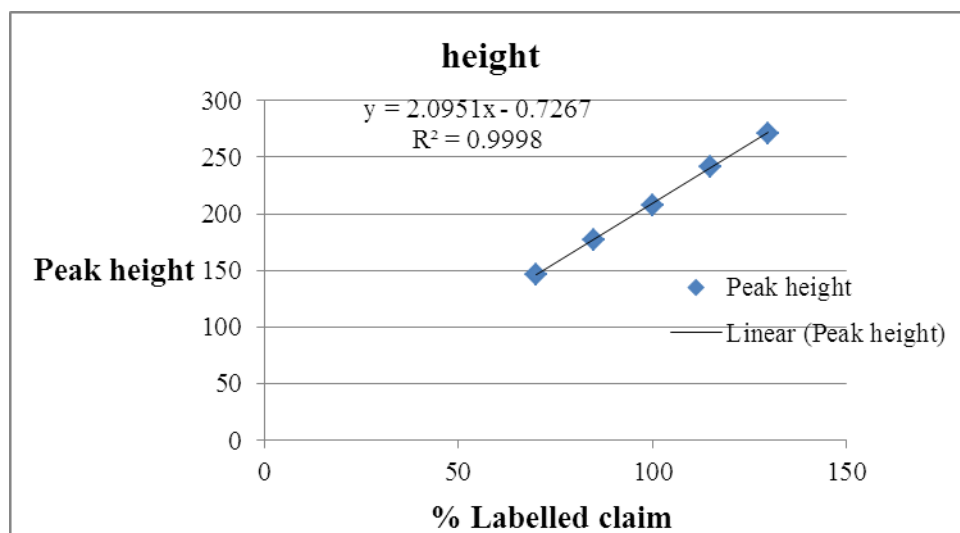
B = amount of drug contributed by pre-analyzed tablet powder (mg)

C = weight of pure drug added (mg)

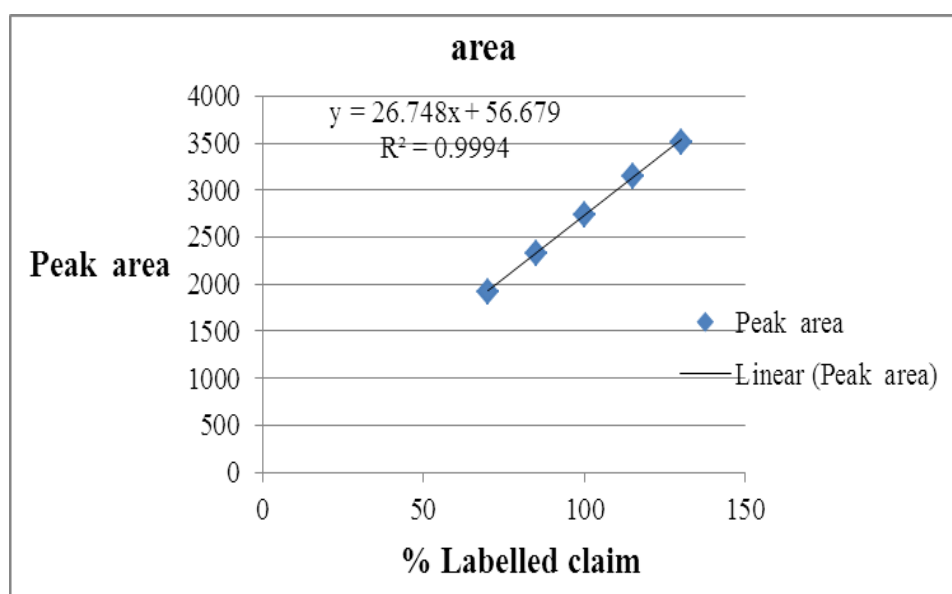
The results of study are given in Table 1.

**Range of method**

A graph was plotted as densitometric response (peak height and area) vs. percent of labeled claim on the basis of accuracy studies data (Fig. 4 a & b).



(a)



(b)

**Fig. 5: Range of Method (a) Peak height (b) Peak area.**

### Robustness

The samples were analysed using proposed method by deliberate small change in the scanning wavelength ( $226 \pm 2$  nm) and mobile phases with different compositions ( $\pm 0.2$  mL) of ethyl acetate: n-hexane: triethyl amine (**4.8: 4.7: 0.5** v/v/v, **5.0: 4.5: 0.5** v/v/v, **5.2: 4.3: 0.5** v/v/v) were used to developed chromatograms. The results of study are given in Table 1.

### Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were determined by the method based on standard deviation of the response and slope of calibration curve as per ICH guidelines.<sup>[7]</sup>

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where  $\sigma$  is the standard deviation of the response (estimated by measuring the response in term of peak height or peak area of standard solution of conc. 100 ng/spot for six times and  $S$  is the slope of calibration curve (obtained from calibration curve). The results of study are given in Table 1.

**Table 1. Results of Validation Parameters for the Proposed HPTLC Method.**

Parameter	Results- Mean $\pm$ SD, % RSD)	
	Peak height	Peak area
Precision:		
1. Repeatability (n=6)	99.40 $\pm$ 0.172, 0.174%	99.81 $\pm$ 0.185, 0.185%
1. Intermediate precision		
a) Intra-day (n=3)	99.62 $\pm$ 0.145, 0.145%	99.87 $\pm$ 0.091, 0.091%
b) Inter-day (n=3)	99.47 $\pm$ 0.488, 0.491%	99.51 $\pm$ 0.505, 0.507%
c) Different analyst (n=3)	99.21 $\pm$ 0.768, 0.774%	99.45 $\pm$ 0.716, 0.720%
Accuracy at 70-130 % of labeled claim (n=5)	99.28 $\pm$ 0.287, 0.289%	99.33 $\pm$ 0.561, 0.683%
Robustness:		
1. Change in wavelength (226 $\pm$ 2 nm)	98.59 $\pm$ 0.073, 0.074%	98.80 $\pm$ 0.072, 0.073%
1. Change in ratio of mobile phase	98.40 $\pm$ 0.480, 0.480%	98.63 $\pm$ 0.610, 0.611%
LOD (ng/spot)	6.947	11.97
LOQ (ng/spot)	21.05	36.28

SD- Standard Deviation, % RSD- Percent Relative Standard Deviation.

## RESULTS AND DISCUSSION

Desvenlafaxine succinate has yielded a well resolved compact spot on pre-coated Silica Gel 60 F<sub>254</sub> TLC aluminum plate of layer thickness 0.2 mm using ethyl acetate: n-hexane: triethyl amine (5.0: 4.5: 0.5 v/v/v) as mobile phase under optimized chromatographic conditions (Fig. 1) and *in situ* spectra of this spot have shown 226 nm as maximum wavelength (Fig. 2). A linear response as a function of concentration was observed over the range of 100-500 ng/spot with correlation coefficient R<sup>2</sup>, 0.991 & 0.999 and regression equation  $y = 274.213x + 4.193$  and  $y = 0.187.954x + 3.436$  by peak area and peak height respectively (Fig. 3 a & b). The results of the repeatability and the intermediate precision were quite reproducible with % RSD value well below 1.0 indicates high level of precision of the proposed method under the conditions studied (Table-1). The recovery studies performed by standard addition method over range of 70-130 % of labeled claim yielded the recovery close to 100% indicating the capability of the method to accurately measure the drug contents free of interference of



excipients. The linear response of the analyte concentration in sample matrix as a function of labeled claim indicates the wide range of accurate measurement of drug content over 70-130% of labeled claim (Fig. 4. a & b). The deliberate small changes in experimental conditions with respect to scanning wavelength and mobile phase composition have no significant effect on the results by the proposed method indicates reasonable robustness of the method (Table-1). The LOD and LOQ values are indicative of sensitivity of the method to detect and to determine the drug content down to few nanograms.

## CONCLUSION

The results of the various validation parameters indicate that the method is quite simple, precise, accurate, sensitive and rapid which may be used for routine assay of Desvenlafaxine in tablet.

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## REFERENCES

1. Sanghavi B. J., Srivastava A. K., “Adsorptive stripping differential pulse voltammetric determination of Venlafaxine and Desvenlafaxine employing Nafion-carbon nanotube composite glassy carbon electrode”, *Electrochim. Acta.*, 2011; 56: 4188- 4196.
2. Perry R., Cassagnol M., “Desvenlafaxine: A New Serotonin Nor-epinephrine Reuptake Inhibitor for the treatment of adults with major depressive disorder”, *Clin. Ther.*, 2009; 31: 1374-1404.
3. Alfinito P. D., Huseltona C., Chena X., Deecher D. C., Pharmacokinetic and pharmacodynamic profiles of the novel serotonin and norepinephrine reuptake inhibitor Desvenlafaxine succinate in ovariectomized Sprague–Dawley rats, *Brain Res.*, 2006; 1098: 71–78.
4. Liu W., Cia H., Li H., “High performance liquid chromatography- electrospray ionization mass spectrometry (HPLC-ESI/MS) method for simultaneous determination of Venlafaxine and its three metabolites in human plasma”, *J. Chromatogr. B.*, 2007; 850: 405-411.

5. Pawar S. M., Khatal L. D., Gabhe S. Y., Dhaneshwar S. R., “LC-UV and LC-MS evaluation of stress degradation behavior of Desvenlafaxine”, JPA., 2012; 2(4): 264-271.
6. Pawar S. M., Dhaneshwar S. R., Application of Stability Indicating High Performance Thin Layer Chromatographic Method for Quantitation of Desvenlafaxine in Pharmaceutical Dosage Form, J. of Liq. Chromatogr. Relat. Technol., 2012; 35: 499-510.
7. ICH, Q2 (R1) Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Geneva, Switzerland, November, 2005.