Advancement and Validation of Stability Representative RP-HPLC Assay Method of Desvenlafaxine In Pure And Pharmaceutical Dosage Form

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ABSTRACT

This paper portrays the advancement and approval of a solidness showing fluid chromatographic strategy for measure of desvenlafaxine in unadulterated and in plans utilizing X-Terra RP C18, 250x4.6 mm, 5 μm particle size column with detection at wavelength 226nm. In the present investigation the mobile phase comprising of sodium dihydrogen orthophosphate buffer (pH 4.0) and acetonitrile in the proportion of 60:40%/v with the Flow rate of 1.0ml/min and column temperature at surrounding temperature uncovered the better resolution and affectability for desvenlafaxine. The maintenance time of desvenlafaxine peak was about 3.632min independently. The created approach be accepted according to ICH regulations which incorporate framework appropriateness, particularity, linearity, exactness, accuracy, vigor, toughness, affectability, cutoff of detection and measurement contemplates and the outcomes were incorporated in this paper.

Keywords: Desvenlafaxine, stability indicating method, RP-HPLC, ICH regulations.

INTRODUCTION

Desvenlafaxine[1](Figure.1) 4-[2-dimethyl amino-1-(1-hydroxy-cyclo hexyl) ethyl] phenol, is an antidepressant drug grouped under the class of nor epinephrine reuptake inhibitors (SNRI), worn to extravagance main depression. It is manufactured and marketed by sun pharmaceuticals as Zyven-OD tablets intended for oral administration (equivalent to Desvenlafaxine 50mg).

A literature survey unconcealed that few analytical strategies (two chromatographical methods)[2,3] were reported for the estimation of desvenlafaxine either on an individual basis or together with alternative medicine. Within the present paper the author tried and developed straightforward, selective stability demonstrating RP-HPLC assay technique for desvenlafaxine within unadulterated and formulations. This research paper describes a straightforward RP-HPLC technique using the RP C-18 X-Terra column (250x4.6mm i.d; particle size 5μm) for the determination of desvenlafaxine in pure and in dose forms. The projected RP-HPLC technique was valid in compliance with International Conference on Harmonization pointers [4].
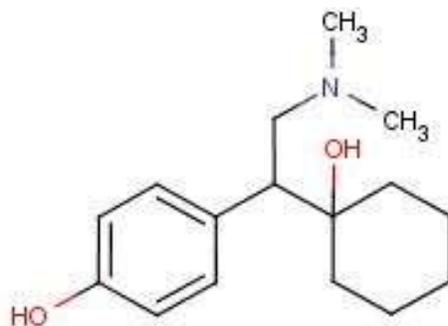

Figure 1: Chemical Structure of Desvenlafaxine

MATERIALS AND METHOD

Instrumentation:

The HPLC analysis of desvenlafaxine was performed on fluid chromatography, Waters division 2996, PDA identifier module furnished with programmed injector with infusion volume 20 μl, and 2693 siphon. C-18 X-Terra segment (250x4.6mm i.d; molecule size 5μm) was utilized as a stationary phase in the present examine. The infusion volume of the test was 20μL. The UV-finder was placed to a wavelength of 226nm and chromatographic runtime was 10minutes. The whole HPLC framework be equilibrated prior to creation of every infusion. Shimadzu balance (BL-220H) was utilized for all gauging.

Chemicals and solvents:

Desvenlafaxine standard (99.9% unadulterated) was acquired as a skilled sample from Laborato,

Mumbai. Oral tablets [in the brand name of Zyven-OD tablets [Label guarantee 50mg of desvenlafaxine] were obtained from the neighborhood drug store. Acetonitrile (HPLC grade), Orthophosphoric acid (GR Grade), Sodium dihydrogen phosphate monohydrate (GR Grade) and Triethylamine (GR Grade) were acquired from Qualigens Ltd., Mumbai. The cleansed water arranged by utilizing a Milli-Q framework was utilized for the readiness of buffer and different aqueous solutions.

Mobile phase preparation:

Set up a filtered and degassed blend of buffer (pH 4.0) and acetonitrile in the proportion of 60:40% v/v was utilized as a mobile phase in the current test individually.

Buffer preparation:

Accurately gauge and move about 2.72gms of Sodium dihydrogen phosphate (monohydrate) and 2.0mL of triethylamine in 1000mL of cleansed water and blend. Change pH to 4.0 (±0.05) with a diluteorthophosphoric acid solution. Filter the arrangement through a 0.45µm membrane filter.

Diluent preparation:

In the present study mobile phase is used as diluent.

Preparation of Standard solution:

Precisely weigh up 100mg of desvenlafaxine, moved into a 100mL volumetric flask at that point, include 60mL of diluent and sonicated to dissolve. Cool the solution to room temperature lastly weakened sufficient with a similar diluent [stock solution]. Move aliquots of the above solution [stock] into a progression of various 100mLvolumetric flasks and weaken to the volume with the diluent individually to acquire working standard solutions of focus ran from $2.0 - 12.0\mu g.mL^{-1}$ separately. 20µL of these solutions were infused in triplicate into HPLC framework and the peak areas were recorded.

Investigation of dosage forms:

Ten tablets of Zyven-OD tablets [Label guarantee 50mg of desvenlafaxine] acquired from the neighborhood drug store were gauged and finely powdered. A precisely gauged bit of the powder, proportionate to about 100mg of desvenlafaxine was moved to a 100mL volumetric flagon pursued by the expansion of 70mL of diluent. The arrangement was sonicated at a controlled temperature for 30min and weakened sufficient with a similar diluent and blended completely. Channel the arrangement all the way through 0.45µm layer channel. Plan diverse working example arrangements in the focus scope of 2.0 to $12.0\mu g.mL^{-1}$ by weakening the above arrangement into a progression of 100mL volumetric jars and weakened to volume with diluent. 20µLof these

arrangements were infused in triplicate into HPLC framework and went before said for the standard individually.

RESULTS AND DISCUSSION:

Method development:

Basic parameters, for example, the wavelength of detection, selection of column and composition of the mobile phase and impact of stream rate on the column were considered in detail in building up this compelling strategy for the measure of desvenlafaxine. To learn the absorbance maxima for the proposed technique working standard arrangement containing desvenlafaxine of fixed fixation was set up in diluent and go through a bright spectrophotometer. In the present examination, the detection wavelength 226nm was chosen by checking drug over a wide scope of wavelength 200 nm to 400nm.

At first, trial preliminaries were utilized distinctive stationary phases like C8 and C18, diverse mobile phases containing buffers like phosphate buffer of pH 4.0 were made. From the above examinations, it was seen that RP C-18 X-Terra column (250x4.6mm i.d; molecule size 5µm) gave the crest with better gaussian shape for desvenlafaxine and observed to be nearly superior to other C8 column and the framework reasonableness information got with this column is appeared in **Table.**

To progress the shape and width of the crest further advancement studies were made and these outcomes affirmed that the sodium dihydrogen orthophosphate buffer (pH-4.0) and acetonitrile in the proportion of 60:40%v/v with the flow rate of 1.0ml/min and surrounding temperature uncovered better goals and affectability for desvenlafaxine. The regular maintenance time of the desvenlafaxine peak was about 3.632 min. individually. The approving chromatogram and the framework appropriateness aftereffects of the present created RP-HPLC strategy for desvenlafaxine were displayed in in Figure-2 and Table-1. separately.

Table 1: System Suitability Limits for Desvenlafaxine

Name of the compound	Retention time	Theoretical plates	Tailing factor	Peak area
Desvenlafaxine	3.632	5978	1.17	2289149

Table 2: Linearity Learnings of Desvenlafaxine

Linearity & Response of Desvenlafaxine		
% Level (approx.)	Concentration (µg/ml)	Peak area ratio
25	2.0	586659
50	4.0	1169032
75	6.0	1734175
100	8.0	2284207

125	10.0	2834626
150	12.0	3379407
Slope, b		278722
Intercept, a		46962
r2		0.9999
LOD (µg/mL)		0.141
LOQ (µg/mL)		0.470

Chromatographic conditions:

The compound was isolated isocratically on a C-18 X-Terra column (250x4.6mm i.d; molecule size 5µm) with a mobile phase comprising of isocratic mobile phase holding sodium dihydrogen orthophosphate buffer (pH 4.0) and acetonitrile [60:40%v/v] was done with the flow rate of 1.2mL/min at encompassing column temperature.

FORCED DEGRADATION**:**

Control sample:

Weigh up daintily powdered not in excess of 20 tablets. Precisely gauge and shift powder proportional to 50mg of desvenlafaxine into 100mL volumetric flask containing 70ml of diluent, sonicated for 30minutes through discontinuous trembling by controlled temperature as well as at long last weakened upto the imprint with methanol and blended. Separated the solution through 0.45µm layer channel. Move 5.0mL of the above solution into a 100ml volumetric flask and diluted to volume by means of a similar diluent.

Acid degradation sample:

 Precisely gauge and move powder proportional to 50mg of desvenlafaxine addicted to a 100mL volumetric flask containing 70mL of diluent, and sonicated for 30minutes with discontinuous trembling at controlled temperature followed by adding 10mL of 5N acid, refluxed for 30min at 60°C, then cooled to room temperature and neutralized with 5N NaOH. At last dilute to volume with diluent and separated through 0.45µm filter. Moved 5.0mL of the above solution into a 100mL volumetric flask and diluted with diluent. 20µLof this solution was infused into the recommended HPLC framework Figure.

Base degradation sample:

Accurately gauge and move powder identical to 50mg of desvenlafaxine into a 100mL volumetric flask containing 70mL of diluent and sonicated for 30minutes with irregular trembling at controlled temperature. Include 10mL of 5N Base (NaOH), refluxed for 30min at 60°C.After that cooled to room temperature, neutralized with 5N Acid (HCl), diluted with diluent and blended, filtered through 0.45µm filter. 5.0mL of the above solution was transferred into a 100mL

volumetric flask, diluted with diluent. 20µLof this solution was infused into the endorsed HPLC framework Figure.

Thermal degradation sample:

Accurately gauged and moved proportional to 50mg of desvenlafaxine into a 100mL volumetric flask. Include 70mL of diluent and sonicated for 30minutes by means of irregular trembling at controlled temperature and adulterated to volume with diluent and blended, separated through 0.45µm filter. 5.0mL of the above solution into a 100mL volumetric flask and diluted through similar diluent. 20µLof this solution was infused into the recommended HPLC framework Figure

From the consequences of the above-said degradation ponders, it was uncovered that no degradation was watched. The chromatograms of desvenlafaxine under different degradation studies were spoken to in Figure and these examinations uncovered the reasonableness of the created RP-HPLC technique to be explicit for desvenlafaxine and its degradation items.

Method validation:

The created RP-HPLC strategy is broadly approved for examine of desvenlafaxine in unadulterated and detailing as per ICH guidelines [4] by utilizing the accompanying parameters.

System appropriateness:

The framework reasonableness factors akin to capacity factor, asymmetry factor, tailing factor and number of theoretical plates were determined. It was seen that every one of the qualities be inside the points of confinement (Table 1). These qualities uncovered the achievability of the created strategy for routine pharmaceutical analysis for desvenlafaxine.

Particularity/selectivity:

The selectivity of the present RP-HPLC strategy was assessed by infusing the fake treatment, clear and unadulterated medication solution into the chromatographic framework under the above said improved chromatographic conditions and their separate chromatograms were recorded. Chromatogram of clear solution demonstrated no peaks at the maintenance time of desvenlafaxine peak showing that the diluent solution utilized in test arrangement does not meddle in the examine of desvenlafaxine. Also, the chromatogram of fake treatment solution demonstrated no peaks on the maintenance time of desvenlafaxine peak uncovering that the fake treatment utilized in test planning didn't meddle the measure of desvenlafaxine in unadulterated and definitions.

Linearity:

Linearity solutions for the technique were set up from desvenlafaxine stock solutions at six fixations levels beginning from 25% to 150% of the focused on level of the examine grouping of desvenlafaxine. Standard solutions containing 2.0-12.0µg/ml of desvenlafaxine in every linearity

level were readied. Alignment diagrams were gotten by means of plotting peak area versus the focus information were treated by least-squares direct regression study, the adjustment charts were observed in the referenced fixations the slopes and correlation coefficients be appeared in Table 2. These outcomes demonstrated that there was a magnificent correlation between the peak area and analyte fixation. The linearity bend of desvenlafaxine was portrayed in Figure 4 and the chromatograms for different convergences of the linearity studies were appeared in figures separately.
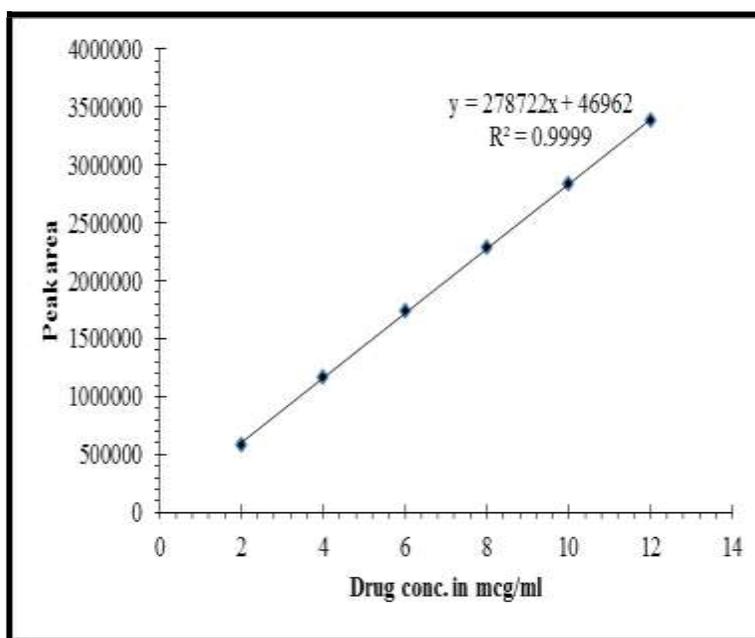

Figure 4: Linearity Curve for Desvenlafaxine

LOD AND LOQ:

The LOD and LOQ values for desvenlafaxine were 0.141 and 0.470µg/ml individually. The consequences of LOD and LOQ were displayed in Table 2 individually which uncovered the affectability of the produced RP-HPLC approach.

Precision:

The accuracy of the current RP-HPLC technique was evaluated by six reproduce infusions of 100% test focus and the outcomes were communicated as far as standard deviation and %RSD. The % RSD values for the peak areas of desvenlafaxine in the investigation of technique exactness were observed to be 0.102 and 0.91% individually and these outcomes showed that the current RP-HPLC strategy was profoundly exact. The framework accuracy results were given in Table 3

Table 3: Values of Method Precision

S.No	Name	RT	Area
1	solution-1	3.633	2259856

2	solution-2	3.6352261025
3	solution-3	3.6392268956
4	solution-4	3.6392268323
5	solution-5	3.6432275468
6	solution-6	3.6412315462
AVG*		3.6382274848
STD DEV*		0.00420702.7
% RSD*		0.1020.91

Accuracy:

The exactness of the proposed technique was dictated by the standard expansion strategy that be executed at three focus levels of 50%, 100%, and 150%. The standard medication solutions be examined within triplicate at every point according to the projected strategy and their separate chromatograms were spoken to in respective figures. The percent recuperation at each level was determined and their outcomes are introduced in Table 4. These outcomes announced the best recuperation i.e.,99.77-100.04% for desvenlafaxine showing that the created RP-HPLC technique was precise.

Table 4: Accuracy Outcomes Of Desvenlafaxine

S.NO	50% AREA	100% AREA	150% AREA
Injection-1	1129136	2203247	3371243
Injection-2	1120356	2198591	3368234
Injection-3	1121024	2253152	3360174
AVG*	1123505	2218330	3366550.333
AMT Recovered*	49.88	100.04	149.48
%Recovery*	99.77	100.04	99.65

*Mean of three determinations considered

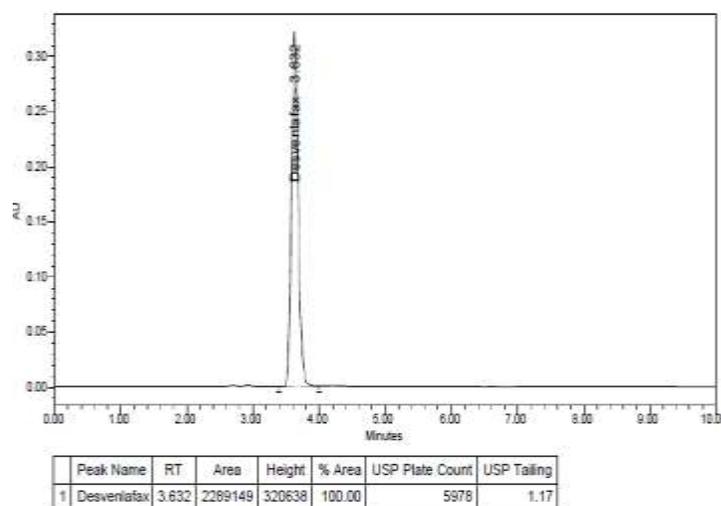| Peak Name	RT	Area	Height	% Area	USP Plate Count	USP Tailing
1 Desvenlafax	3.632	2289149	320638	100.00	5978	1.17

Figure: 2 Typical HPLC Chromatogram of Desvenlafaxine

Robustness:

The vigor of the present proposed strategy was finished by adjusting the trial conditions that incorporate (I) the impact of progress in stream rate and (ii) the impact of column temperature. The stream pace of the mobile phase in the present measure was 1.0 mL/min. To ponder the impact of the stream pace on the resolution, the stream rate was changed by ±0.2 units (0.8 and 1.2mL/min) and column temperature lying on elution be learned at 33°C and 37°C rather than encompassing temperature. In all the above said differed conditions, the segments of the mobile phase stayed consistent.

The aftereffects of the power investigation of the proposed strategy are displayed in Table 5 and these outcomes demonstrated that the examine estimation of the test readiness solution was marginally influenced and was as per that of real during the above- said change conditions. And in addition, the framework reasonableness parameters were likewise discovered a tasteful end that the created strategy as hearty.

Table 5: Results of Robustness Study

Robust conditions		Desvenlafaxine Theoretical plates	RT	Peak Area
Flow rate	1.0 ml/min	5521	3.287	2067378
	1.4 ml/min	6586	4.093	2580385
Temperature	33°C	6006	3.652	2303305
	37°C	6066	3.626	2279768

Roughness:

The toughness of the proposed RP-HPLC technique was assessed by two unique experts with various instruments in a similar research facility. The % RSD of pinnacle zones of desvenlafaxine was determined and the trial results are appeared in Table 6. These outcomes uncovered that the %RSD was inside the breaking points demonstrating that the created RP-HPLC strategy was observed to be rough.

Table 6: Results of Ruggedness Studies Of Desvenlafaxine

| S no	Name	Analyst-1 Area	Analyst -2 Area
1	injection-1	2259856	2121024
2	injection-2	2261025	2129549
3	injection-3	2268956	2150425
4	injection-4	2268323	2191218
5	injection-5	2275468	2208786
6	injection-6	2315462	2189784
	AVG*	2274848	2165131

STD DEV*	20702.7	36391.8
% RSD*	0.91	1.681

Analysis of pharmaceutical formulations:

Analysis of promoted tablets {Zyven-OD tablets [Label guarantee 50mg of desvenlafaxine]} was completed utilizing the above said streamlined mobile phase and HPLC conditions. The % medication substance of tablets acquired by the proposed strategy for desvenlafaxine was observed to be 99.99% separately. This demonstrated the estimation of measurements structures was precise inside the acknowledgment level. The outcomes are given in Table.7.

Table 7: Study of Marketed Tablets [D-Veniz]

Drug	Label claim	Quantity found	%assay
Zyven-OD tablets [Label claim 50mg of desvenlafaxine]	50mg	49.99	99.99
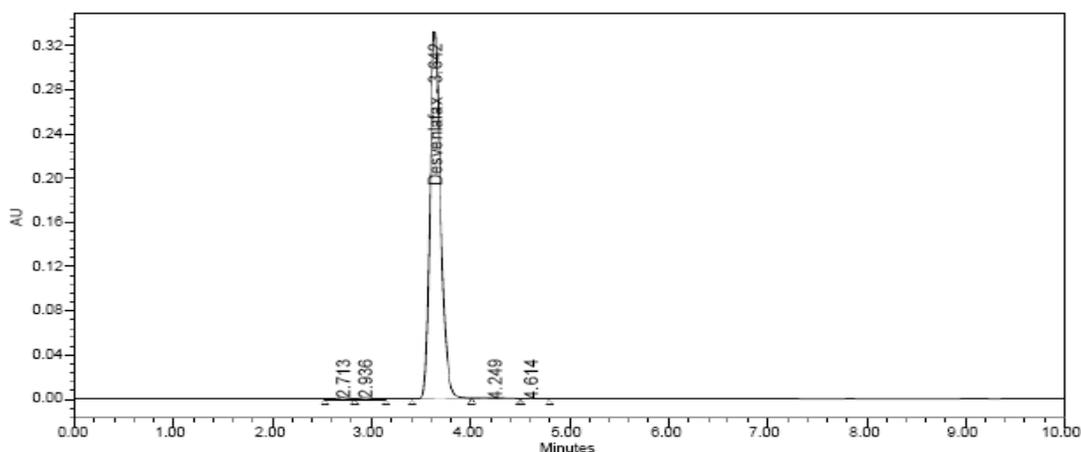
	Peak Name	RT	Area	Height	% Area	USP Plate Count	USP Resolution	USP Tailing
1		2.713	12641	1888	0.40	3598		0.94
2		2.936	10909	1700	0.37	4990	1.26	1.43
3	Desvenlafax	3.642	2085644	322627	98.93	5419	3.76	1.19
4		4.249	13436	765	0.21	1094	1.64	1.03
5		4.614	2581	355	0.09	8985	0.99	1.53

Figure:3. A - Chromatograph of Desvenlafaxine in Acidic Hydrolysis
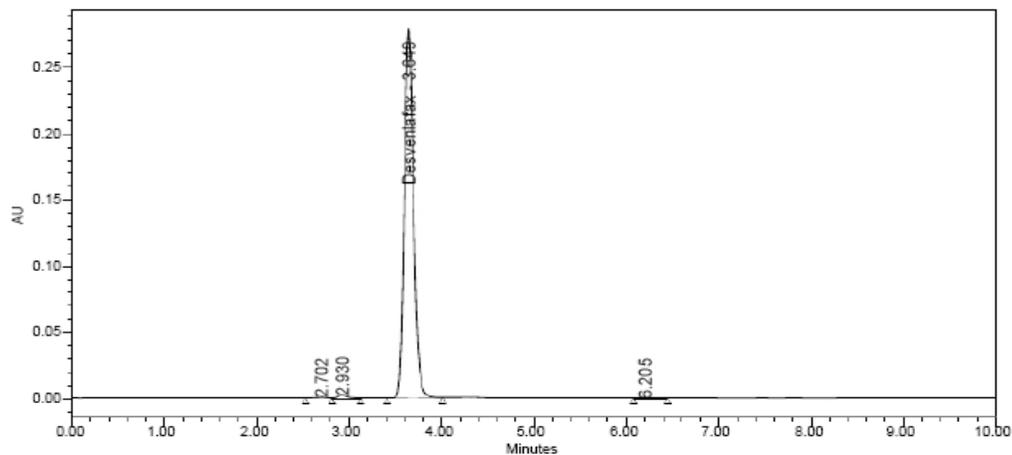
	Peak Name	RT	Area	Height	% Area	USP Plate Count	USP Resolution	USP Tailing
1		2.702	4090	1606	0.71	3568		0.89
2		2.930	12709	1933	0.85	4659	1.26	1.25
3	Desvenlafax	3.649	1946924	278064	98.21	6257	3.90	1.16
4		6.205	327	129	0.23	7173	10.76	1.42

Figure:3. B - Chromatograph of Desvenlafaxine in Basic Hydrolysis
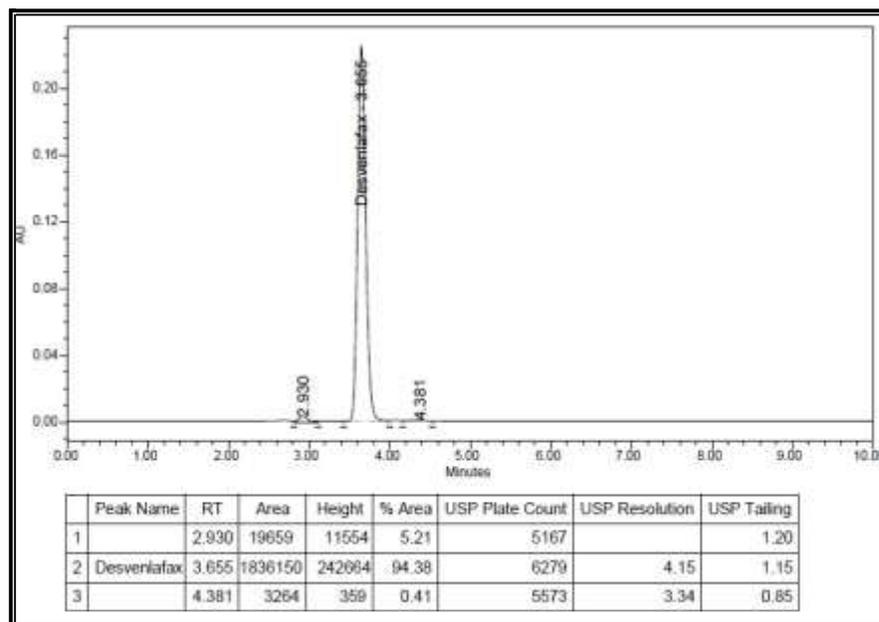
	Peak Name	RT	Area	Height	% Area	USP Plate Count	USP Resolution	USP Tailing
1		2.930	19659	11554	5.21	5167		1.20
2	Desvenlafax	3.655	1836150	242664	94.38	6279	4.15	1.15
3		4.381	3264	359	0.41	5573	3.34	0.85

Figure: 3.C - Chromatograph of Desvenlafaxine In Thermal Degradation

CONCLUSIONS:

In the proposed investigation, the factual analysis demonstrated that the technique was exact, and repeatable. The aftereffects of stress studies showed the reasonableness of the proposed technique for desvenlafaxine under different forced degradation environments viz. acid, base, dry warmth, unbiased, photolytic and UV degradation. All degradation items framed during pressure studies were very much isolated from one another just as analyte peak exhibiting that the created

technique was explicit and steadiness demonstrating nature uncovering that the created RP HPLC strategy be able to utilized for habitual investigations of desvenlafaxine.

ACKNOWLEDGEMENTS:

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