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Research Article

QUANTIFICATION OF POTENTIAL IMPURITIES BY A STABILITY INDICATING HPLC METHOD IN DESVENLAFAXINE SUCCINATE MONOHYDRATE ACTIVE PHARMACEUTICAL INGREDIENT

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ABSTRACT

A sensitive, selective and stability indicating reverse phase method has been developed for the quantitative determination of potential impurities of Desvenlafaxine succinate monohydrate active pharmaceutical ingredient. Efficient chromatographic separation was achived on C8 stationary phase. Forced degradation study confirmed that the newly developed method was specific and selective to the degradation products. Major degradation of the drug substance was found to occur under oxidative stress conditions to form desvenlafaxine *N*-oxide. The method was validated according to ICH guidelines with respect to specificity, precision, linearity and accuracy. Regression coefficient value was greater than 0.999 for Desvenlafaxine succinate monohydrate impurities. Detection limit of impurities were in the range of 0.002-0.016% indicating the high sensitivity of the newly developed method. Accuracy of the method was established based on the recovery obtained between 94.65% and 101.05% for all impurities.

Keywords: Desvenlafaxine succinate monohydrate, validation, impurity, Forced degradation.

1. INTRODUCTION

Desvenlafaxine succinate monohydrate is chemically Rs-4-[2-dimethylamino-1-(1-hydroxycyclohexy) ethyl] phenol succinate monohydrate[1]. Desvenlafaxine succinate monohydrate is used for the treatment of patients with major depressive disorder. Depression is one among the most rampant form of psychiatric disorders and a leading cause for morbidity and mortality[2]. Desvenlafaxine succinate monohydrate is major active metabolite of venlafaxine and is categorized as a serotonin norepinephrine reuptake inhibitor (SNRI)[3]. A very few methods have been reported in literature for the analysis of Desvenlafaxine succinate monohydrate bulk drug samples. There are HPLC methods reported for the estimation of Desvenlafaxine succinate monohydrate in pharmaceutical formulations. Several methods have been reported in the literature for simultaneous determination of Desvenlfaxine succinate monohydrate with venlafaxine in plasma and biological fluids using HPLC coupled to spectrophotometric[4-7] and spectrofluorimetric detection[8-11]. Capillary electrophoresis[12-18], LC-MS/MS[19-24] and HPLC-ESI/MS[25] have been used as well. LC methods were not reported in any of the pharmacopoeias. Extensive literature survey reveals that no reference exists for the quantitative determination of impurities by a stability indicating HPLC method and characterization of unknown impurities of Desvenlafaxine succinate monohydrate drug substance. Hence it was felt necessary to develop an accurate, rapid, selective and sensitive stability indicating LC method for the determination of Desvenlafaxine succinate monohydrate impurities. Objective of current study was to develop a significant method and

also to carry out method validation and forced degradation study of desvenlafaxine succinate monohydrate.
Chemical structures of Desvenlafaxine succinate monohydrate and potential impurities are shown in figure-1 to figure-4.

1. EXPERIMENTAL

1.1 Materials and reagents

Samples of Desvenlafaxine succinate monohydrate API and standards of impurity-A, Impurity-B and Desvenlafaxine *N*-oxide impurity-C were obtained from Chemical Research and Development department, Troy Life sciences Bangalore. Deionized water was prepared using milli-Q plus water from Millipore (Bedford, USA). HPLC grade methanol, acetonitrile, analytical reagents potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, hydrogen peroxide was purchased from Merck India Limited (Mumbai, India).

1.2 Equipements and Chromatographic conditions

The HPLC system consisted of a Shimadzu prominence separate module LC-10ADVP equipped with dual wavelength absorbance and PDA detector water Empower-2 software was used for the data acquition and processing. Phenomenex Luna C8 HPLC column of 250 mm X 4.6 mm id, 5µm particle size was used. The column was kept at 35°C. Mobile phase A consisted, 10mM potassium dihydrogen phosphate in water adjusted pH 5.0 ± 0.05 with sodium hydroxide solution and mobile phase B consisted acetonitrile-water (90:10 v/v) in gradient mode (T_{min} A:B) $T_0$95:5, T_{20}80:20, T_{30}60:40, T_{40}40:60, T_{45}95:5, T_{55}95:5. The flow rate was set to 1.0ml/min with detector wavelength fixed at 225nm. The injection volume was 20ul for a sample concentration of 1mg/ml prepared in diluents (mobile phase: Acetonitrile, 80:20 v/v).

1.3 Sample preparation for forced degradation studies

Stress degradation studies were performed as per ICH guidelines Q1 (R2) to demonstrate the stability indicating nature and specificity of the proposed method. About 50mg of desvenlafaxine succinate monohydrate sample were transferred into 3 different 50ml volumetric flasks and subjected to forced degradation studies under acidic (1N HCl for 3h) and basic conditions (1N NaOH for 3h). The stressed samples of acid and base degradation were neutralized with NaOH and HCl respectively and made up to volume with diluents. Oxidative degradation was carried out using 10% hydrogen peroxide (80°C for 3h). Solid state stability of the drug substance was carried out by (a) thermal degradation at 105°C for 24h and (b) photolytic degradation was performed by keeping 1g of each solid sample in two separate loss on drying bottles (LOD bottles). In photo stability chamber, samples were exposed to get a minimum exposure of 1.2million lux hours for light and 200W h/m^2 for ultra violet region. Samples were withdrawn at appropriate time and subjected to LC analysis, using (1mg/ml) concentration.

1.4 Preparation of stock solutions for method validation

A test preparation of 1mg/ml of Desvenlafaxine succinate monohydrate API sample was prepared by dissolving in diluent (buffer-acetonitrile, 80:20). A stock solution of impurities was prepared by dissolving 15mg each impurity of impurity-A, impurity-B and impurity-C in diluents and made up to 10ml with diluents. 1ml of each individual stock solution was transferred into a 100ml volumetric flask and made up to volume with diluent. From this stock solution standard solution of 0.0015mg/ml of each impurity and 0.0015mg/ml of Desvenlafaxine succinate monohydrate were prepared. This standard solution was also used for checking solution stability and robustness parameters.

1.5 NMR spectroscopy

^{1}H NMR spectra were recorded at 400 MH_Z, using Bruker 400MH_Z spectrometer (Bruker, Falladen, Switzerland) equipped with a 5 mm BBO probe and Z-gradient shim system. The ^{1}H spectra were recorded with 1s pulse repetition time using 30° flip angle. Samples were dissolved in deteriorated chloroform. The ^{1}H chemical shift values were reported on the δ scale in ppm relative to TMS. All spectra were recorded with sample spinning.

1.6 Mass Spectrometry

The Mass spectrometry studies were performed on Agilent LC/MS Quadruple 6180 using electrospary ionization source, atmospheric pressure ionization. The typical electrospary source conditions were spray voltage, 5Kv, capillary voltage, 15-20V, heated capillary temperature 250°C, and tube lens offset voltage 20V, sheath gas (N_2) pressure, 20 psi.

2. RESULTS AND DISCUSSION

2.1 Method development

The main objective of method development was to achieve efficient separation between Desvenlafaxine succinate monohydrate and known impurities. The resolution between impurity-A and impurity-B were poor when different stationary phase like C18 and other brand columns were used in different mobile phases containing buffers like di-sodium hydrogen phosphate, ammonium acetate and ammonium di-hydrogen phosphate or combination of these with pH 4 to 6 using organic modifiers such as methanol and acetonitrile. Use of Luna Phenomenex C8 column, acetonitrile-water mixture as mobile phase –B and column temperature 35°C were significant in achieving the desired resolution of impurity-A and impurity-B. Optimum resolution between impurity-A and impurity-B were achieved when the pH of buffer was adjusted to 5.0. After several trials for gradient profile, chromatographic conditions were finalized as described under section high performance liquid chromatography.

2.2 Forced degradation studies and identification of major degradants

Desvenlafaxine succinate monohydrate was found to be stable to acid and base condition. In oxidative degradation condition significant degradation was observed and the impurity formed at RRT 1.1 was identified as desvenlafaxine *N*-oxide. Chromatograms of the forced degradation study have been depicted from fig.6-8 and table 1 shows the proton NMR characterization data of impurities. Degradation studies and peak purity test results derived from PDA detector confirmed that the spectral purity of desvenlafaxine succinate monohydrate peak was homogenous, thus, confirming the stability indicating power of the newly developed method.

3. METHOD VALIDATION

Validation study was carried out for the analysis of impurity-A, impurity-B and impurity-C. The system suitability and selectivity were checked by injecting 1.0mg/ml of desvenlafaxine succinate monohydrate solution containing 0.15% of all impurities (fig 5). Method validation results are summarized in Table-2.

3.1 Accuracy and precision

Accuracy of the method was evaluated in triplicates at six concentration levels, i.e. from QL-150% of the analyte concentration (1.0mg/ml). The percentage of recovery for each impurity was calculated at each level and found in the range of 94.65-101.05% (Table2). The precision of the related substances method was checked by injecting six individual preparations of (1.0mg/ml) desvenlafaxine succinate monohydrate spiked with 0.15% of each impurity. Percentage RSD for peak areas of each impurity were calculated (Table 2). Precision was also determined by performing the same procedures on a different day.

3.2 Quantitation limit and detection limit

The quantitation limit and detection limit for all the impurities were determined by signal to noise ratio method. The QL for impurity-A, impurity-B and impurity-C were about 0.006, 0.007 and 0.05% respectively. The DL for impurity-A, impurity-B and impurity-C were about 0.002, 0.002 and 0.016% respectively, indicating high sensitive of the method.

3.3 Linearity and Range

Linearity was established between ranges of QL to 0.3% of the analyte concentration (1.0mg/ml). The correlation coefficient obtained was not less than 0.999 for all impurities. Standard deviation of peak area was significantly low and %RSD was below 3.0%.

3.4 Robustness and ruggedness

Close observation of analysis results of the deliberately changed in chromatographic conditions (flow rate, and column temperature) revealed that the resolution between impurity-A and impurity-B was greater than 2.0. The intermediate precision (ruggedness) of the method was also evaluated by a different analyst and different analyst and different instrument in the same laboratory with %RSD areas of each impurity within 5.0. The experimental data are shown in table 3.

3.5 Solution stability

The solution stability of desvenlafaxine succinate monohydrate and its related substances was established by spiked sample solution. The solution stability experiments data confirmed that sample solutions were stable up to 48 hrs.

4. CONCLUSION

The newly developed RP-LC method for quantitative determination of desvenlafaxine succinate monohydrate and related substances

was found to be sensitive, precise, accurate, specific and stability indicating. The major oxidative degradant was identified as desvenlafaxine *N*-oxide. Thus, newly developed method has been validated as per regulatory requirements and can be used for routine and stability studies for the quantitative determination of potential impurities in desvenlafaxine succinate monohydrate drug substances.

Table 1: Comparative study of ^{1}H NMR

Compound	Solvent	1H NMR
Impurity-A	CDCl3	δ0.9-1.7 (m, 10H), δ 2.6 (s, 3H), δ 2.8 (s, 3H,), δ 3.8 (s, 3H), δ 2.6 (s, 3H), δ 3.2-3.4 (m, 2H), δ 4.1 (dd, 1H), δ 6.8 (d, 2H), δ 7.2 (d, 2H)
Impurity-B	CDCl3	δ 1.4-2.0 (m, 8H), δ 2.8 (m, 5H), δ 2.6 (s, 3H), δ 3.4-3.5 (m, 3H), δ 6.7 (d, 2H), δ 6.9 (d, 2H)
Impurity-C	MeOD	δ 0.9-1.6 (m, 10H), δ 2.8 (s, 3H), δ 2.9 (s, 3H), δ 3.1-3.3 (m, 2H), δ 3.7 (dd, 1H), δ 6.7 (d, 2H), δ 7.1 (d, 2H)

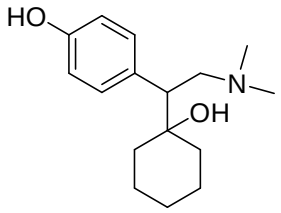

Fig. 1: Desvenlafaxine Succinate monohydrate

Table 2: Method validation summary report

Parameter	Impurity-A	Impurity-B	Impurity-C
System Suitability			
RT	32.203	31.725	26.222
RRT	1.36	1.34	1.10
Linearity (r)	0.9993	0.9990	0.9991
Quantitation limit (%)	0.006	0.007	0.05
Detection limit (%)	0.002	0.002	0.016
Precision at QL (RSD)	3.55	1.58	1.80
% Recovery at QL (n=3)	102.74	106.37	100.53
Accuracy (% Recovery)			
50%	100.12	100.26	100.0
75%	99.30	101.05	96.51
100%	95.61	99.33	98.89
125%	94.21	100.14	97.47
150%	94.65	98.90	100.34

n, number of determinations; RT, retention time; RRT, relative retention time; R^2, correlation coefficient

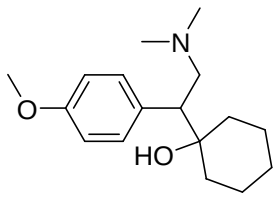

Fig. 2: 1-(2-(dimethylamino)-1-(4-methoxyphenyl) ethyl) cyclohexanol. (Impurity-A)

Table 3: Robustness of the LC method

Validation Parameter	Resolution between Impurity-A and Impurity-B
Flow rate (ml/min)	
0.9	2.65
1.0	2.60
1.1	2.32
Column temperature (°C)	
30	2.26
35	2.65
40	2.72

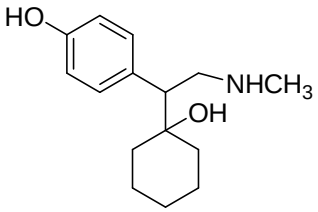

Fig. 3: 4-(1-(1-hydroxycyclohexyl)-2-(methylamino) ethyl) phenol (Impurity-B)

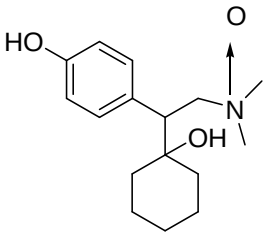

Fig. 4: Desvenlafaxine *N*-Oxide (Impurity-C)

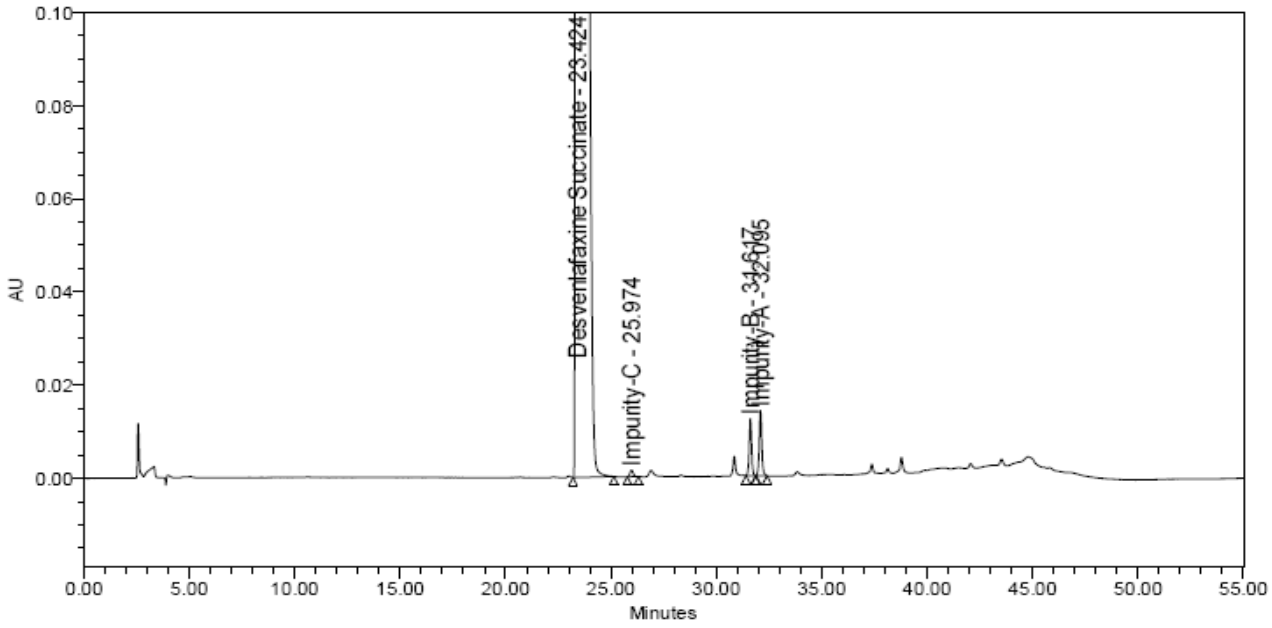

Fig. 5: Typical Chromatogram of impurity spiked Solution in Desvenlafaxine succinate monohydrate

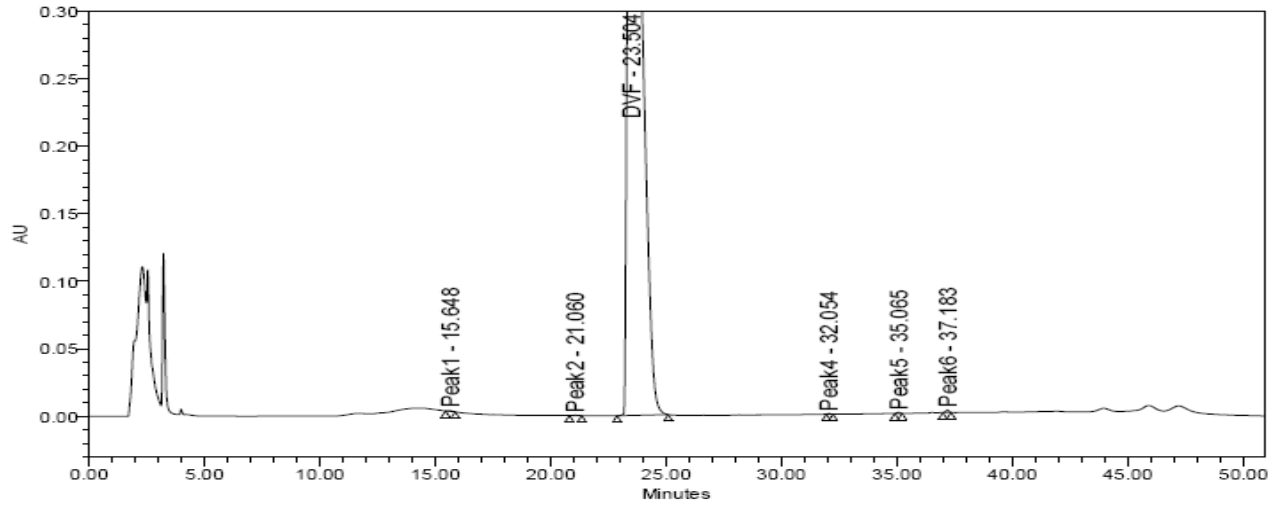

Fig. 6: Typical Chromatogram of Desvenlafaxine succinate monohydrate in 1N NaoH

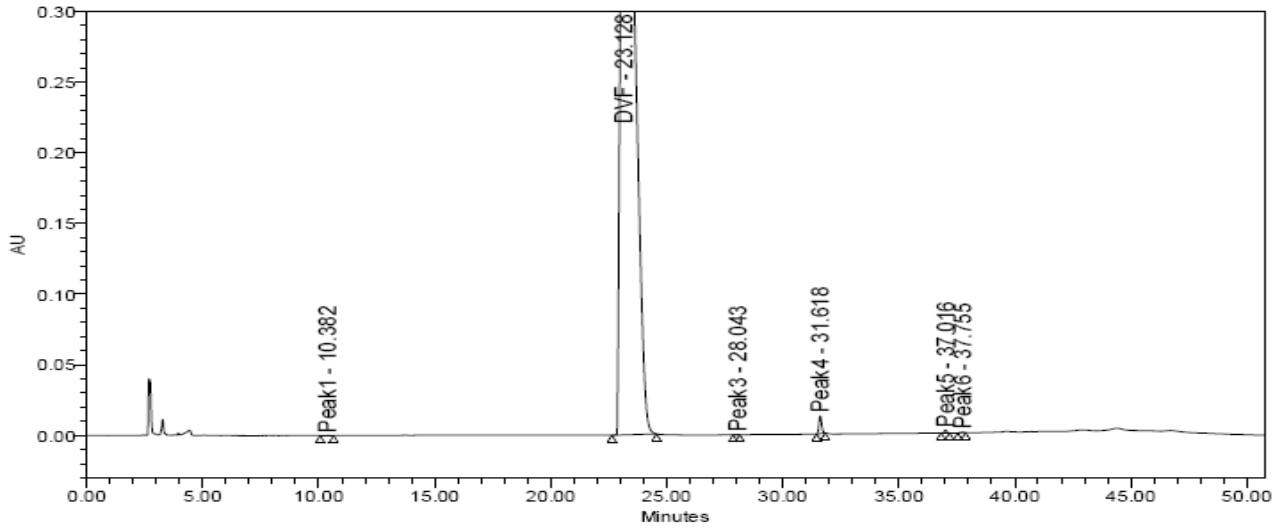

Fig. 7: Typical Chromatogram of Desvenlafaxine succinate monohydrate in 1N HCl

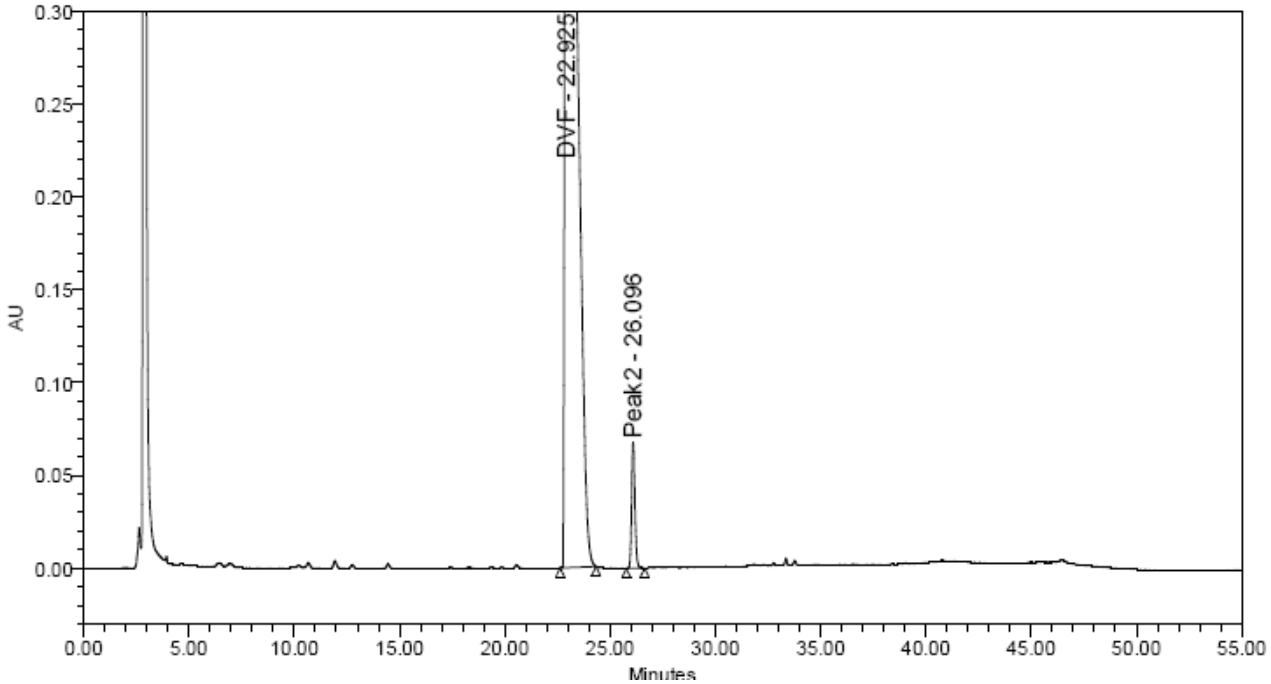

Fig. 8: Typical Chromatogram of Desvenlafaxine succinate monohydrate in 10% H_2O_2

5. ACKNOWLEDGEMENT

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6. REFERENCES

1. Deecher DC, Beyer CE, and Johnston M. A new serotonin and norepinephrine reuptake inhibitor. J Pharmacol Exp Ther. 2006; 318: 657-665.
2. Ashwani Arya and Tarun Kumar. Depression: Review. J Chem Pharma Res. 2011; 3(2): 444-453.
3. Richard PP and Manouchkathe CP. Desvenlafaxine A new serotonin-nor-epinephrine reuptake inhibitor for the treatment of adults with major depressive disorder. Clin Therap. 2009; 31: 1374-1404.
4. Raut BB, Kolte BL, Deo AA, Bagool MA, and Shinde D.B. Development and validation of a rapid HPLC-fluresence method for simultaneous determination of venlafaxine and its major metabolite in human plasma. J Liq Chromatogram Related Technol. 2003; 26(8): 1297-2001.
5. Matoga M, Pehoureq F, Titier K, Dumora F, and Jarry C. Rapid high-performance liquid chromatographic measurement of venlafaxine and O-desmethylvenlafaxine in human plasma, application to management of acute intoxications. J Chromatogr Related Technol. 2001; 760(2): 213-218.
6. Tournel G, Houdret N, Hedouin V, Deveau M, Gosset, D, and Lhermitte M. High-performance liquid chromatographic method to screen and quantitative seven selective serotonin reuptake inhibitors in human serum. J Chromatogr B Biomed Sci Appl. 2001; 761(2): 147-158.
7. Hicks DR, Wolaniuk D, Russell A, Cavanaugh N, and Kraml M. A high performance liquid chromatographic method for the simultaneous determination of venlafaxine and O-desmethylvenlafaxine in biological fluids. Ther Drug Monit. 1994; 16(1): 100-107.
8. Ardakani KH, Foroumadi A, and Rouini MR. Development and validation of a rapid HPLC-fluorescence method for simultaneous determination of venlafaxine and its major metabolites in human plasma. DARU, J Pharma sci. 2010; 18(2): 97-102.
9. Roberto M, Laura M, Roberta C, Salvatore F, Maria AR, and Mario A. Analysis of the second generation antidepressant venlafaxine and its main active metabolite O-desmethyl venlafaxine in human plasma by HPLC with spectrofluorimtric detection. J Chromatogr B. 2007; 856(1-2): 88-94.
10. Waschgler R, Moll W, Konig P, and Conca A, Quantification of venlafaxine and O-desmethylvenlafaxine in human serum using HPLC analysis. Int J Cli Pharmacol Ther. 2004; 42: 724-728.
11. Vu RL, Helmeste D, Albers L, and Resist C. Rapid determination of venlafaxine and O-desmethyl venlafaxine in human plasma by high-performance liquid chromatography with fluorimetric detection. J Chromatogr B Biomed Sci Appl. 1997; 703: 195-201.
12. Labat L, Deveaux M, Dallet P, and Dubost JP. Separation of new antidepressants and their metabolites by micellar electro kinetic capillary chromatography. J Chromatogr B Analyst Technol Biomed Life Sci. 2002; 773(1): 17-23.
13. Desiderio C, Aturki Z, and Fanali S. Use of vancomycin silica stationary plane in packed capillary electro chromatography I. Enantiomer separation of basic compounds. Electrophoresis. 2001; 22(3): 535-543.
14. Fanali S, Rudaz S, Veuthey, JL, and Desiderio C. Use of vancomycin silica stationary plane in packed capillary electro chromatography II. Enantiomer separation of venlafaxine and 0-desmethylvenlafaxine in human plasma, J Chromatogr A. 2001; 919(1): 195-230.
15. Rudaz S, Stella C, Balant-Gorgia E, Fanali S, and Veuthey, JL. Simultaneous stereoselective analysis of venlafaxine and O-desmethylvenlafaxine enantiomer in clinical samples by capillary electrophoresis using changed cyclodextrine. J Pharma Biomed Anal. 2000; 23(1): 107-115.
16. Rudaz S, Veuthey JL, Desiderio C, and Fanali S. Enantioseparation of venlafaxine and O-

desmethylvenlafaxine by capillary electrophoresis with mixed cyclodextrins. Chromatographia. 1999; 50(5-6): 369-372.
17. Clement EM, Odontiadias J, and Franklin M.J. Simultaneous measurement of venlafaxine and its major metabolite, oxydesmethylvenlafaxine in human plasma by high performance liquid chromatography with coulometer detection and utilization of solid-phase extraction. Chromatogr B. 1998; 705: 303-308.
18. Fanali S, Cotichini V, and Porra RJ. Analysis of venlafaxine by capillary zone electrophoresis. Capillary Electrophor. 1997; 4(1): 21-26.
19. Shah GR, Thaker, BT, Surati KR, and Prabia MH. Simultaneous determination of venlafaxine and its main metabolite O-desmethyl venlafaxine in rat plasma by LC-MS/MS. Anal Sci. 2009; 25: 1207-1210.
20. Rajassekhar D, Kumar IJ, and Venkateswarlu P, Rapid high-performance liquid chromatography-tandem mass spectrometry method for simultaneous measurement of venlafaxine and O-desmethylvenlafaxine in human plasma and its application in comparative and bioavailability study. Biomed Chromatogr. 2009; 23: 1300-7.
21. Castro AM, Concheiro Quintela O, Cruz A, and Lopez-Rivadulla M. LC-MS/MS method for the determination of nine antidepressants and some of their main metabolites in oral fluid and plasma. Study of correlation between venlafaxine concentrations in both matrices. J pharm Biomed Anal. 2008; 48:183-93.
22. Patel B.N, Sharma N, Sanyal M, and Shrivastav PS., Liquid chromatography tandem mass spectrometry assay for the simultaneous determination of venlafaxine and O-desmethylvenlafaxine in human plasma and its application to a bioequivalence study. J Pharma Biomed Anal. 2008; 47: 603-611.
23. Castaing N, Titier K, Receveur-Daurel M, Le-Deodic M, Le-Bars D, Moore N, and Molimard M. Quantification of eight new antidepressants and five of their active metabolites in whole blood by high-performance liquid chromatography-tandem mass spectrometry. J Anal Toxicol. 2007; 31: 334-41.
24. Bhatt J, Jangid A, Venkatesh G, Subbaiah G, and Singh SJ. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for simultaneous determination of venlafaxine and its active metabolite O-desmethyl venlafaxine in human plasma. Chromatogr B Analy Technol Biomed Life Sci. 2005; 829: 75-81.
25. Liu W, Cai HL, and Li HD. High performance liquid chromatography-electro spry ionization Mass spectrometry (HPLC-MS/ESI) method for the simultaneous determination of venlafaxine and its three metabolities in human plasma. J Chromatogr B analyt Technol Biomed Life Sci, 2007; 850: 405-411.