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R E S E A R C H A R T I C L E

Development and validation of a Sensitive Bio analytical method for the quantitative estimation of Desvenlafaxine in human plasma samples by LC–MS/MS: Application to bioequivalence study

Narendra Kumar Reddy Kolli*[1], Swetha Polagani[1], Nageswara Rao B[1]

[1]*Sri Vani School of Pharmacy, Chevuturu, Vijayawada, AP. India.*

A B S T R A C T

A Novel sensitive and selective ultra-high performance liquid chromatography-coupled to mass spectroscopy (LCMS/MS) method was developed and validated for the quantification of Desvenlafaxine in human plasma ,when Desvenlafaxine D6 used as internal standard. HPLC analysis was carried out on Thermo-BDS Hypersil 3µ,C8 50*4.6 mm with mobile phase 5 ml Ammonium Acetate : Methanol (20:80) (v/v) and flow rate of 0.800 mL/min. Method development comprises of:-Tuning parameters of the Analyte , ISTD Optimization of Source parameters and Mass parameters Optimization of Chromatographic conditions, Optimization of extraction procedure (LPE). System Suitability, Mobile Phase stability: Accuracy , Precision and specified analytical method for quantification were found to give accurate and precise results within the range of Desvenlafaxine 1.002ng/mL to 1000.165 ng/mL, the specified analytical methods for quantification of Desvenlafaxine were found to give reproducible results when samples are re-injected. Stock solutions of Desvenlafaxine and DesvenlafaxineD6 were stable for 20 hours 19minutes when kept at room temperature. The specified analytical method for quantification of Desvenlafaxine was found to have freeze and thaw stability for 05 cycles for Desvenlafaxine in biological matrix containing K2EDTA human plasma as the anticoagulant .Desvenlafaxine is found to be meeting the acceptance criteria for stability when kept on bench top for 18hours 25mins in the biological matrix containing K2EDTA as an anticoagulant.

Keywords: Desvenlafaxine, Desvenlafaxine D6, Human K2EDTA plasma, LC-MS/MS, 5mL Ammonium Acetate: Methanol (20:80) (v/v).

A R T I C L E I N F O

***Corresponding Author**
Narendra Kumar Reddy Kolli
Sri Vani School of Pharmacy,
Chevuturu, Vijayawada, AP. India.
MS-ID: IJMPR4523

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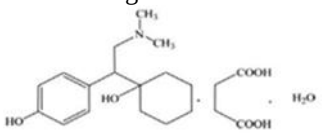
C O N T E N T S

1. Introduction

Desvenlafaxine (DVS) was an novel serotonin (5-HT) that was currently in clinical development for the treatment of major depressive disorder and vasomotor symptoms associated with menopause. Desvenlafaxine was originally synthesized as part of research project to discover structural anlongs of 4-(2-(Dimethyl amino)-1-(hydroxycyclohexy) ethyl phenol is the major active metabolite of the antidepressant, a medication used to treat major depressive, generalized anxiety and panic disorders. Literature survey revealed that few LC-MS methods have been reported for estimation of DVS in biological matrices.



Chemical Structure of Desvenlafaxine

Several analytical methods have been reported for the determination of Desvenlafaxine such as reverse-phase HPLC, LC-MS and a degration pat way for drug proposed. The purpose of the present study was to develop and validate an LC-MS/MS method as per ICH Bioanalytical method validation Guidlines, with simple sample preparation technique to determine Desvenlafaxine concentration in human plasma and apply it to a bioequivalence study of Desvenlafaxine tablet. This assy method demonstrated acceptable sensitivity (LLOQ: 1.002ng/ml) Precision ,accuracy, selective recovery and stability, less absolute and reported method for desvenlafxine utilizes Desvenlaflaxine D_6 as internal standard For liquid-liquid extraction of analyte and internal. HPLC injection and ionization variability, it is recommended to use a deuterated/stable isotope analyte. The present study utilizes deuterated Desvenlafaxine D_6 as a internal standard which has advantage over the other reported methods.

2. Materials and Methods

Instumentation:

Shimadzu SIL-HTC HPLC System and applied biosystems (AB sciex) LC-MS/MS API 3200.

Reagents/materials:

Methanol (HPLC gade), Ammonium Acetate (GR/AR), Methyl tertiary buyl ether –MTBE-(HPLC grade) or MilliQ, Desvenlafaxine and Desvenlafaxine D_6 Internal standard.

Stock, standard and sample solutions:

All stocks, stock dilutions, spiking solutions, spiked in matrix and all solutions used were prepared as per STP.

Biological Matrix: Homogeneous K2EDTA human plasma (MV035/PM/01/12) obtained by pooling screend K2EDTA human plasma was used as blank for analysis during method, validation, selectivity and sensitivity tests were performed before bulk spiking.

Calibration curve (CC) standards and quality control sample concentrations: Four calibration curves covering the entire range of the analytical method for Desvenlafaxine from 1.002ng/ml to 1000.165ng/ml was analyzed each with eight concentration levels.

3. Results and Discussion

Table 1: Pure drugs and excipients

Injection Number	Retention Time(min)		Area Ratio
	Desvenlafaxine	Desvenlafaxine D6	
1	1.32	1.32	28.185
2	1.32	1.32	27.644
3	1.32	1.32	28.026
4	1.32	1.33	27.912
5	1.31	1.32	28.155
6	1.32	1.32	27.493
Average	1.318	1.322	27.9025
Standard Deviation	0.0041	0.0041	0.28051
CV (%)	0.3	0.3	1.0

Table No 2: Results for Desvenlafaxine (Hemolytic)

Hemolytic Plasma ID	Desvenlafaxine			Desvenlafaxine D6		
	Response in Blank	Response in LLOQ	% Interference	Response in Blank	Response in LLOQ	% Interference
HPM/127/11	0	1667	0.00	0	53241	0.0
Total No. of Matrices	1	Number of Matrices Meeting the Requirements				1
Percentage of Matrices Meeting Selectivity Criteria				100.0%		

Table No 3: Results of Auto Sampler Carryover

Sample Name	Area at the RT of Desvenlafaxine	%Carry Over of Desvenlafaxine	Area at the RT of Desvenlafaxine D6	%Carry Over of ISTD
Mobile phase/ Reconstitution Solution-I	0	0.0	0	0.0
Mobile phase/ Reconstitution Solution-II	0	0.0	0	0.0
Mobile phase/ Reconstitution Solution-III	0	0.0	0	0.0
Mobile phase/ Reconstitution Solution-IV	0	0.0	0	0.0
Lowest Aqueous standard(AQSLLOQ)	1849		67383	
Extracted blank-I	0	0.0	0	0.0
Extracted blank-II	0	0.0	0	0.0
Extracted blank-III	0	0.0	0	0.0
Lowest Extracted standard (Ext LLOQ)	1620		47112	

Table No 4: Results of Auto Sampler Carryover

Sample Name	Area at the RT of Desvenlafaxine	%Carry Over of Desvenlafaxine	Area at the RT of Desvenlafaxine D6	%Carry Over of ISTD
Mobile phase/Reconstitution Solution-I	0	0.0	0	0.0
Mobile phase/Reconstitution Solution-II	252	6.9	0	0.0
Mobile phase/Reconstitution Solution-III	0	0.0	0	0.0
Mobile phase/Reconstitution Solution-IV	0	0.0	0	0.0
Lowest Aqueous standard(AQSLLOQ)	3627		185870	
Extracted blank-I	0	0.0	0	0.0
Extracted blank-II	0	0.0	0	0.0
Extracted blank-III	0	0.0	0	0.0
Lowest Extracted standard(Ext LLOQ)	2517		111076	

Table No 5: Results for Desvenlafaxine

Plasma ID	Desvenlafaxine			Desvenlafaxine D6		
	Response in Blank	Response in LLOQ	% Interference	Response in Blank	Response in LLOQ	% Interference
HPM/030/12	288	1837	16.5	0	51875	0.0
HPM/042/12	0	1960	0.0	0	51777	0.0
HPM/048/12	128	1864	7.3	0	51826	0.0
HPM/066/12	149	1636	8.5	0	51298	0.0
HPM/067/12	0	1520	0.0	0	51570	0.0
HPM/075/12	0	1673	0.0	0	51864	0.0
Average		1748.3			51701.7	
Total No. of Matrices	6	Number of Matrices Meeting the Requirements				6
Percentage of Matrices Meeting Selectivity Criteria				100.0%		

Table No 6: Results for Desvenlafaxine (Lipemic)

Lipemic Plasma ID	Desvenlafaxine			Desvenlafaxine D6		
	Response in Blank	Response in LLOQ	% Interference	Response in Blank	Response in LLOQ	% Interference
HPM/128/11	0	1265	0.0	0	52589	0.0
Total No. of Matrices	1	Number of Matrices Meeting the Requirements				1
Percentage of Matrices Meeting Selectivity Criteria				100.0%		

Observation for Desvenlafaxine:

Precision: The coefficient of variation (CV) or relative standard deviation (RSD) of the back-calculated concentrations for the stability samples of HQC is 3.1 % and 2.1 % for LQC samples. The coefficient of variation (CV) or relative standard deviation (RSD) of the back-calculated concentrations for the fresh samples of HQC is 3.4 % and 3.2% for LQC samples.

Accuracy compared with nominal concentrations:

The mean of the back-calculated concentrations of the fresh HQC samples is 97.8 % and for fresh LQC samples is 103.6 %.The mean of the back calculated concentrations of the HQC stability samples are 97.7 % and for LQC stability samples is 108.6 %. The % Stability of HQC samples is 99.9% and 104.9 % for LQC samples. LC-MS/MS Method for estimation of Desvenflaxine in human plasma was developed and validated using Desvenlafaxine D_6 as an internal standad (IS) .Sample preparation was accomplished by Liquid-Liquid extraction .The processed samples were chromatographed on advance high purity C_{18} 50 X 4.6 mm, 5u colomuns using a mobile phase containg a mixture of methanol, Ammonium acetate, Methyl tertiary butyl ether and water .The method was validated over a concentration range of 1.002ng/ml to 1000.165ng/ml concentrations for Desvenlafaxine .During validation selectivity and recovery exercise was carried out .Precision and accuracy batches. Results of various stabilities, reinjection reproducibility and ruggedness were carried out.

4. Conclusion

The LC–MS/MS validated method proved to be very simple, sensitive and reliable and successfully applied for the pharmacokinetic study in human plasma. The assay method is specific due to the inherent selectivity of tandem mass spectrometry. The major advantage of this method is the use of Desvenlafaxine D_6 as an internal standard. The specified analytical method for quantification of Desvenlafaxine was found to have freeze and thaw stability for 05 cycles for Desvenlafaxine in biological matrix containing K2EDTA human plasma as the anticoagulant .Desvenlafaxine is found to be meeting the acceptance criteria for stability when kept on bench top for 18hours 25mins in the biological matrix containing K2EDTA as an anticoagulant. This method is very suitable and convenient for pharmacokinetic and bioavailability studies of the drug Desvenlafaxine.

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