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Spectroflourimetric determination of venlafaxine hydrochloride and o-desmethylvenlafaxine in marketed formulations

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ABSTRACT

A simple, sensitive, precise and reproducible spectrofluorimetric method for the analysis of venlafaxine hydrochloride and O-desmethylvenlafaxine in pharmaceutical formulations has been developed. Venlafaxine hydrochloride and O-desmethylvenlafaxine are synthetic novel antidepressant drugs, and acts by inhibiting the reuptake of serotonin and noradrenaline. Venlafaxine hydrochloride exhibits maximum fluorescence intensity at excitation wavelength of 274nm and emission wavelength 610 nm, and similarly O-desmethylvenlafaxine at excitation wavelength 226 nm and emission wavelength 300 nm respectively. Beer's law was obeyed for both Venlafaxine and O-desmethylvenlafaxine in the concentration range of 50-300 ng/ml and 10-600 ng/ml respectively. The limit of detection and limit of quantitation for venlafaxine and O-desmethylvenlafaxine were found to be 7.72 ng/ml, 23.40 ng/ml and 1.95 ng/ml, 5.91 ng/ml respectively. This simple, sensitive and precise method hence can be successfully employed for the routine determination of venlafaxine and O-desmethylvenlafaxine in various pharmaceutical preparations.

Key words: Venlafaxine hydrochloride, O-desmethylvenlafaxine, recovery, marketed formulation, spectrofluorimetry.

INTRODUCTION

Venlafaxine hydrochloride (VEN), (1-[2-dimethylamino)-1-(4-methoxy phenyl) ethyl] cyclohexanol) hydrochloride is a third generation antidepressant. The drug is a potent inhibitor of neuronal serotonin and norepinephrine reuptake, but a weak inhibitor of dopamine reuptake [1-2]. VEN has several metabolites (Fig. 1), one of which is biologically active. About 1% of VEN is desmethylated to N-desmethylvenlafaxine (NDV), 1-[2-(methylamino)-1-(4-methoxyphenyl)ethyl] cyclohexanol; 16% becomes N,O-didesmethylvenlafaxine (NODV), 4-[2-(methylamino)-1-(1-hydroxycyclohexyl)ethyl]phenol; and 56% is metabolized to O-desmethylvenlafaxine (ODV), 4-[2-(dimethylamino)-1-(1-hydroxycyclohexyl)ethyl]phenol which, unlike others, has an activity profile on monoamine transporters similar to VEN [3-4].

Literature reviewed reveals various analytical methods like spectrophotometric analysis, RP-HPLC method for determination of VEN in dosage forms and bulk [5-6]. Moreover, several analytical methods like HPLC with fluorimetric detection, HPLC-ESI-MS, LC-tandem MS have been reported in literature for analysis of VEN in biological fluids like human plasma, milk, serum etc. [7-13].

Similarly, for ODV various analytical methods like RP-HPLC [14], HPTLC [15], stability indicating methodology by HPLC and HPTLC have been reported [16-17]. Also HPLC method has been reported for analysis of ODV in various biological fluids like human serum, human plasma, rat plasma, dog plasma, mouse plasma etc. [18-20].

Literature reveals no separate spectrofluorimetric method for determination of VEN and ODV in formulation. Hence, in the present article, a simple, precise, and sensitive spectrofluorimetric method for the determination of VEN and ODV in pure form and in extended release formulation was developed.

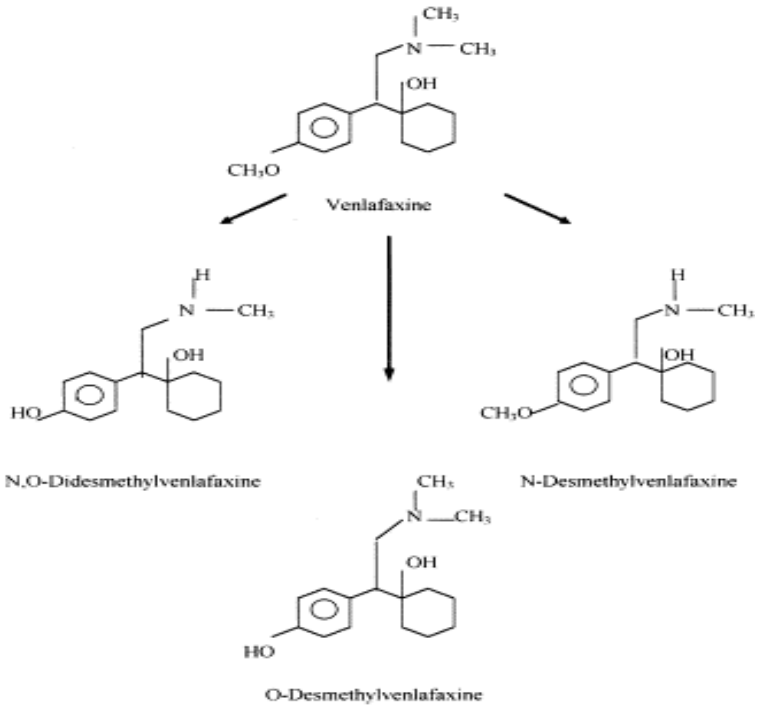

Fig. 1: Molecular structure of venlafaxine and its metabolites

MATERIALS AND METHODS

Reagents and Materials

VEN and ODV were procured from Torrent Research Centre, Ahmedabad, India and Zydus Research Centre, Ahmedabad, India respectively. All chemicals were of analytical grade and procured from Merck Pvt. Ltd., Mumbai. All the solutions were freshly prepared in 0.05 M sulphuric acid. The commercial fixed dose formulation procured from the market for analysis of VEN are VENTAB 37.5mg, 75mg, VENLIFT 37.5mg, 75mg and VENIZ XR 37.5mg, 75mg and for ODV are D-VENIZ 50mg, NEWVEN 50mg, and MDD-XR 50mg.

Instrumentation

The fluorescence intensity was measured using a Spectrofluorimeter (RF 5301 Model Shimadzu, Japan) equipped with xenon lamp and a 10 nm quartz cell. The excitation and emission wavelength band passes were both set at 10 nm. All the assays were performed at room temperature, excitation and emission wavelength were set at 274 and 610 nm for VEN and 226 nm and 300 nm for ODV respectively.

Preparation of standard drug solution

The standard VEN and ODV (10 mg) were weighed accurately and transferred to 100ml volumetric flask and diluted with 0.05 M sulphuric acid up to the mark to a final concentration of 100 µg/ml. Working standard solution of lower concentration (300 ng/ml) were prepared by further dilution of the above standard stock solution with 0.05M sulphuric acid for both the drugs.

Calibration curve for VEN and ODV

Different aliquots of working standard solutions were transferred into a series of serially numbered 10 ml volumetric flasks to prepare a final concentration in the range of 50-300 ng/ml of VEN and 10-600 ng/ml for ODV. The solutions were further diluted to 10 ml with 0.05M sulphuric acid. The fluorescence intensity of the solution of VEN and ODV were measured at excitation wavelength 274 nm, emission wavelength 610 nm and excitation wavelength 226 nm, emission wavelength 300 nm respectively using 0.05 M sulphuric acid as a blank. The fluorescence intensity of the VEN and ODV solutions were measured for different concentrations. The calibration curve was prepared by plotting Relative Fluorescence Intensity (R.F.I) versus concentration of drugs for both VEN and ODV respectively.

Analysis of marketed formulation

Twenty tablets were powdered and weighed accurately. The quantity of powder equivalent to 10 mg of VEN and 10 mg of ODV were weighed differently and dissolved in 100 ml 0.05M sulphuric acid. The solution was filtered through Whatman filter paper No 42 (GE Healthcare UK Limited, UK). Further dilutions were made up to 10 ml with 0.05M sulphuric acid in volumetric flask to prepare a final concentration of 100 ng/ml. The content of the VEN and ODV present in different marketed formulations was calculated from the calibration graph.

Validation of analytical method

The methods were validated according to International Conference on Harmonization guidelines for validation of analytical procedures [21]. Statistical analysis was performed using Microsoft Excel [22-23].

RESULTS AND DISCUSSION

Validation of the method

Various analytical method validation parameters for VEN and ODV are as summarized in Table 1.

Linearity

Calibration curves of VEN and ODV were linear over the concentration range of 50-300 ng/ml and 10-600 ng/ml respectively, which is as good as that reported in literature.

Sensitivity

The detection limit for VEN and ODV (LOD=3.3*SDa/b, where SDa is the standard deviation of intercept and b is the slope of the regression line) was 7.72 ng/ml and 1.95 ng/ml respectively. The quantification limit (LOQ=10*SDa/b) for VEN and ODV was 23.40 ng/ml and 5.91 ng/ml respectively.

Table-1: Summary of various parameters for VEN and ODV

Parameters	Venlafaxine hydrochloride	O-desmethylvenlafaxine
Beer's law limit	50-300 ng/ml	10-600 ng/ml
Linear regression equation[a]	F=0.1019x-0.1478	F=1.1393x-11.53
Correlation coefficient(r)	0.9954	0.9965
S.D. of intercept(S_a)	0.2384	0.675
S.D. of slope(S_b)	0.0017	0.0035
Limit of Detection (ng/ml)	7.72	1.95
Limit of Quantitation (ng/ml)	23.40	5.19

Precision

The intraday precision assays were carried out through replicate analysis (n=5) corresponding to 50, 100 and 150 ng/ml and 100, 150, 200 ng/ml for proposed method for VEN and ODV respectively. The interday precision was also evaluated through replicate analysis (n=3) for three consecutive days at the same concentration levels as used in interday precision for VEN and ODV respectively. The results of these assays are reported in Table 2 for VEN and Table 3 for ODV. RSD values for intraday and interday precision is less than 2%, assuring good precision of the method.

Table-2: Intra and Interday Precision studies for VEN

Precision	Concentration (ng/ml)		RSD	SEM[b]	C.I[c]
	Amount taken	Amount found[a] ± S.D.			
Intraday (n=5)	50	53.82±0.17	0.032	0.077	0.216
	100	98.9±0.20	0.020	0.091	0.255
	150	146.69±0.40	0.027	0.179	0.497
Interday (n=9) 3 days	50	54.61±0.086	0.016	0.049	0.066
	100	96.81±0.33	0.034	0.190	0.253
	150	147.37±0.15	0.010	0.088	0.117

[a] Mean, [b]SEM.-Standard error of mean,

[c] C.I., confidence interval at 95% confidence level (intraday t=2.776, four degrees of freedom),(interday t=2.306, eight degrees of freedom)

Table-3: Intra and Interday Precision studies for ODV

Precision	Concentration (ng/ml)		RSD	SEM [b]	C.I [c]
	Amount taken	Amount found [a]± S.D.			
Intraday (n=5)	100	99.71±0.80	0.0061	0.358	0.99
	150	150.3±1.28	0.0083	0.573	1.59
	200	201.35±1.89	0.0086	0.846	2.35
Interday (n=9) 3 days	100	97.85±1.48	0.011	0.856	1.14
	150	151.37±2.04	0.013	1.181	1.57
	200	198.90±1.19	0.005	0.692	0.92

[a] Mean, [b]SEM.-Standard error of mean,
[c] C.I., confidence interval at 95% confidence level (intraday t=2.776, four degrees of freedom),(interday t=2.306, eight degrees of freedom)

Accuracy and recovery

Accuracy of the developed method for estimation of VEN and ODV was assessed by spiking with standard at three different concentration levels, 50, 100 and 150 %. The results of recovery studies for different formulations are as shown in Table 4 for VEN and Table 5 for ODV respectively.

Table 4: Recovery studies for VEN

Formulation	Amount of powder weighed equivalent to (mg)	Amount of VEN standard added	Percentage Recovery ± S.D	SEM[a]	C.I[b]
	10	50%	99.89±0.45	0.259	1.118
VENLIFT	10	100%	101.80±0.56	0.325	1.399
37.5 mg	10	150%	95.02±0.76	0.441	1.901
	10	50%	104.68±1.04	0.605	2.605
VENLIFT	10	100%	102.87±1.06	0.615	2.649
75 mg	10	150%	102.49±0.43	0.251	1.081
	10	50%	95.7±0.30	0.177	0.763
VANIZ	10	100%	97.52±1.02	0.590	2.542
37.5 mg	10	150%	96.94±1.11	0.643	2.767
	10	50%	106.65±1.80	1.040	4.47
VANIZ	10	100%	96.83±0.81	0.469	2.02
75 mg	10	150%	93.38±0.69	0.399	1.71
	10	50%	98.03±0.53	0.309	1.33
VENTAB	10	100%	104.16±0.58	0.335	1.44
37.5 mg	10	150%	92.03±1.41	0.815	3.51
	10	50%	97.8±0.50	0.288	1.24
VENTAB	10	100%	95.47±0.58	0.336	1.44
75 mg	10	150%	97.33±0.97	0.565	2.43

a= standard error of mean, b= C.I., Confidence Interval at 95% confidence level and two degree of freedom. (t=4.303)

Table 5: Recovery studies for ODV

Formulation	Amount of powder weighed equivalent to (mg)	Amount of ODV standard added	Percentage Recovery ± S.D	SEM [a]	C.I [b]
	10	50%	96.74±1.44	0.831	3.57
D-VENIZ	10	100%	101.96±2.70	1.562	6.75
50 mg	10	150%	97.3±2.75	1.592	6.85
	10	50%	103.09±0.91	0.526	2.26
NEWVEN	10	100%	93.96±1.88	1.08	4.68
50	10	150%	95.86±1.18	0.682	2.93
	10	50%	98.64±1.03	0.597	2.57
MDD-XR	10	100%	97.7±1.03	0.595	2.56
50 mg	10	150%	101.12±1.59	0.919	3.95

a= standard error of mean, b= C.I., Confidence Interval at 95% confidence level and two degree of freedom. (t=4.303)

Application of proposed method for analysis of formulation

The results of the developed method for the determination of VEN and ODV in commercial marketed formulations are as shown in Table 6 for VEN and 7 for ODV respectively.

Table 6: Determination of Venlafaxine hydrochloride in marketed formulations

Formulation (Tablet/Capsule)	Label claim (mg)	Amount estimated (mg)	% of VEN found ± S.D	%RSD
VENIZ XR capsule	37.5	37.26	99.37 ± 0.014	0.145
	75	75.16	100.22± 0.064	0.631
VENLIFT OD capsule	37.5	37.24	99.30 ± 0.038	0.381
	75	76.05	101.40 ± 0.039	0.382
VENTAB tablet	37.5	36.96	98.98 ± 0.114	1.143
	75	74.28	99.04 ± 0.100	0.999

Table 7: Determination of O-desmethylvenlafaxine hydrochloride in marketed formulations

Formulation (tablet)	Label claim (mg)	Amount estimated (mg)	% of ODV found ± SD	%RSD
D-VENIZ	50	48.25	96.56 ± 2.06	1.624
NEWVEN	50	53.12	106.26± 1.64	1.216
MDD-XR	50	49.64	99.2± 1.15	0.881

CONCLUSION

A sensitive, precise and rapid method was developed for the determination of VEN and ODV in marketed formulation using spectrofluorimetry. Distinct advantages of the method include the simplicity and rapidity of sample preparation, good sensitivity and cost effective methodology. The accuracy has been validated and was found to be in the range of 98.98-101.40 % w/w for VEN and 96.56-106.26 %w/w for ODV. The results of precision studies show that the method is reproducible and precise. This reported method hence can be used for routine quality control of marketed formulation.

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