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Development and Validation of UV – Spectrophotometric Method for Simultaneous Estimation of Venlafaxine Hydrochloride and Bupropion Hydrochloride in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

In the present work, a new simple, precise, specific, accurate and economical UV-Spectrophotometric method for simultaneous estimation of Venlafaxine HCl and Bupropion HCl in bulk and tablet dosage form was developed and validated. Methanol and water were used as a solvent. The analytical wavelengths for Venlafaxine HCl and Bupropion HCl were found to be 223 nm and 249 nm respectively. The developed method was validated as per the ICH guidelines in terms of Linearity and Range, Specificity, Precision, Accuracy, Sensitivity and Ruggedness. The linearity was obtained in the concentration range of 4-24µg/ml for both Venlafaxine HCl and Bupropion HCl with correlation coefficient 0.9995 and 0.9991 respectively. The % RSD for intraday precision and inter-day precision of Venlafaxine HCl was found to be 0.39 and 0.33 respectively and for Bupropion HCl it was found to be 0.27 and 0.15 respectively. In both, the cases values were within the acceptance limit of less than 2%. The mean percent recovery for Venlafaxine HCl and Bupropion HCl were found to be 100.36 to 100.81 and 100.49 to 101.40 respectively. Based on the results obtained the proposed method can be regarded as simple, precise, accurate, reliable, cost effective and can be used for routine quality control of Venlafaxine HCl and Bupropion HCl in bulk and its tablet dosage forms.

INTRODUCTION

Venlafaxine hydrochloride (VENLA) [1, 2] is a novel synthetic antidepressant derivative of ethyl cyclohexanol, metabolized into O-desmethylvenlafaxine and potentiates the activity of CNS. It is referred to as Serotonin norepinephrine reuptake inhibitor (SNRI), because it inhibits the uptake of both noradrenaline (NA) and 5-hydroxytryptamine (5-HT) but, in contrast to older tricyclic antidepressants (TCAs), does not interact with the cholinergic, adrenergic or histaminergic receptors or have sedative properly. It may improve the mood, energy level and help to restore the interest of daily living. The combination effects of the reuptake mechanisms are responsible for the antidepressant action of the drug. VENLA is practically soluble in methanol and partially soluble in the water. The chemical structure of VENLA is **(Figure 1).**

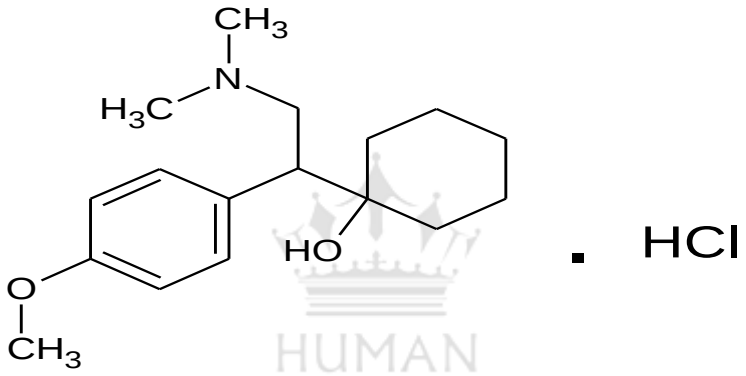


Figure No. 1: Chemical structure of Venlafaxine hydrochloride

Bupropion hydrochloride (BUPRO) [3, 4] is the salt of an aminoketone showing antidepressant activity and potentially used in smoking cessation. The mechanism of the antidepressant effect of Bupropion hydrochloride is unknown. This antidepressant agent has different neurochemical properties from common tricyclic antidepressant. Bupropion hydrochloride is also a selective inhibitor of the neuronal reuptake of noradrenaline and dopamine (catecholamine's) with minimal effect on the reuptake of indolamine (serotonin) and there is no inhibitory effect on monoamine oxidase. It is a weak blocker of the neuronal uptake of serotonin, dopamine and norepinephrine as well as central nicotinic acetylcholine receptor antagonist. BUPRO is practically soluble in water, alcohol and methanol. The chemical structure of BUPRO is **(Figure 2).**

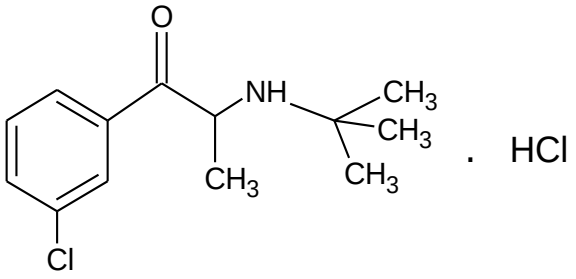


Figure No. 2: Chemical structure of Bupropion hydrochloride

Depression is a mood disorder that causes a persistent feeling of sadness and loss of interest. It is also called major depressive disorder [MDD] or clinical depression. It is linked to the problems or imbalances in the brain with neurotransmitters such as serotonin, norepinephrine and dopamine. Serotonin is produced by the serotonergic neurons. This serotonin neurotransmitter is controlling many body functions such as sleep, aggression, sexual behavior and mood. The decrease in the production of serotonin by its neurons causes the depression.

Bupropion is a USFDA approved unicyclic antidepressant agent which is selective inhibitor of the neuronal reuptake of norepinephrine. It has modest noradrenergic, dopaminergic activity with little serotonergic effect.

Venlafaxine hydrochloride is a novel phenylethylamine serotonin-norepinephrine reuptake inhibitor and it is effective in the treatment of depression.

As compared to monotherapy of Venlafaxine hydrochloride and Bupropion hydrochloride, its combination therapy shows synergistic action on antidepressant activity. This combination shows some unique pharmacological profiles, which effects in the treatment of depression and converts partial response to full response in the patients with treatment-resistant depression. So, it's considered that this combination would reduce the depressive symptoms in the patients, who were unresponsive to various classes of psychotropic agents.

On literature survey, it was found that several methods like UV Spectrophotometric method [5-7], Reverse Phase High Performance Chromatographic method (RP-HPLC) [8, 9] and High-Performance Thin Layer Chromatographic method (HPTLC) [10, 11] for individual drug has been established for Venlafaxine hydrochloride. Bupropion hydrochloride was estimated and validated by UV Spectrophotometric method [12-16], Potentiometric method, Conductometric method [15], Reverse Phase High Performance Liquid Chromatographic

method (RP-HPLC) [16-21] for individual drug, RP-HPLC with other combination of drug has been established for Bupropion hydrochloride [22, 23]. Hence, there is a need for the development of newer, simpler, rapid, accurate and reproducible analytical methods for simultaneous estimation of Venlafaxine hydrochloride and Bupropion hydrochloride in bulk and pharmaceutical dosage forms.

MATERIALS AND METHODS

Instruments used:

Electronic analytical balance, Shimadzu-1800 UV-Spectrophotometer, Ultrasonic bath Sonicator was used in the study.

Reagents and chemicals:

VENLA and BUPRO standards were obtained as gift sample from Angel Biopharmaceutics, Gujarat and Apotex Drugs and PVT.LTD. Bengaluru. All the chemicals were of AR grade and are obtained from the stores of Government College of Pharmacy, Bengaluru. VENLA and BUPRO tablet dosage forms were produced from local pharmacy store.

Selection of solvents:

By carrying out solubility profile study and literature survey, it was found that VENLA and BUPRO are easily soluble in methanol and water. Hence methanol and water were chosen for the UV-Spectrophotometer analysis of VENLA and BUPRO.

Preparation of standards stock solutions:

10 mg of each standard VENLA and BUPRO were weighed separately, into two 100 mL volumetric flask and dissolve in methanol and water respectively to get the concentration of 100µg/ml.

Selection of analytical wavelength:

From the above standard stock solution, 0.4 mL was taken separately into 10 mL volumetric flask and diluted with the solvents and these solutions were scanned in the UV region of 200-400 nm. Maximum absorbance was seen in the wavelength of 223 nm for VENLA and 249

nm for BUPRO. Hence all absorbance measurements were made at 223 nm for VENLA and 249 nm for BUPRO.

Calibration curve:

A series of dilution were prepared from the standard stock solution of VENLA and BUPRO to the concentration range of 4-24 µg/mL for both the drugs. Absorbance of the above solution was measured at 223 nm and 249 nm for VENLA and BUPRO respectively and calibration curve of the absorbance against concentration was plotted and regression coefficient (R^2) was also determined.

Determination of absorptive coefficients:

The absorptive coefficient of both the drugs (VENLA and BUPRO) was determined at selected wavelengths by using the formula **$A=A\ (^{1\%}1cm)\ b \times c$.** Where, c = concentration of the absorbing species, and b = path length in cm. The absorptivity values are then substituted in the following equations (1) and (2):

$$\mathbf{A_1 = ax1\ Cx + ay1\ Cy \ldots\ldots\ldots\ldots\ (1)}$$

$$\mathbf{A_2 = ax2\ Cx + ay2\ Cy \ldots\ldots\ldots\ldots\ (2)}$$

Where,

A_1 and A_2 are absorbance of the sample at 223 nm and 249 nm respectively. ax1 and ax2 are absorptivity's of VENLA at 223nm and 249nm respectively. ay1 and ay2 are the absorptivity's of BUPRO at 223nm and 249nm respectively. Cx and Cy are the concentration of VENLA and BUPRO respectively.

Preparation of sample solution:

Average weight of twenty tablets containing 150 mg of both VENLA and BUPRO (labeled claim) was calculated separately. The tablets were powdered well in glass mortar with pestle. Quantity of powder equivalent to 10 mg of both the VENLA and BUPRO was weighed accurately and transferred separately into 100 mL volumetric flasks. Then the volume was made up with the methanol and water up to 100 mL and filtered through a 0.45 µm membrane filter. From this, 0.4 mL were taken and diluted with methanol and water to 10 mL

of volumetric flask and absorbance was measured at 223 nm and 249 nm against methanol and water as a blank. The assay was performed in triplicate.

Analysis of tablet dosage form:

The above stock solution was diluted with the solvents and absorbance was measured at appropriate wavelength and the concentration of the two drugs were determined using equations (3) and (4). Analysis was done in triplicates.

$$Cx = (A_2\, ay1 - A_1\, ay2) / (ax2\, ay1 - ax1\, ay2) \ldots\ldots\ldots (3)$$

$$Cy = (A_1\, ax2 - A_2\, ax1) / (ax2\, ay1 - ax1\, ay2) \ldots\ldots\ldots (4)$$

METHOD VALIDATION

The developed UV-Spectrophotometric method was developed as per ICH guidelines in terms of linearity and range, specificity, precision, sensitivity, ruggedness and accuracy.

Linearity and range in order to determine Linearity range for developed method a series of solutions of VENLA and BUPRO were prepared using standard stock solution at concentration range of 4-24 µg/mL for both the drugs. The absorbances of resultant solution were measured at 223 nm and 249 nm against methanol and water as a blank. The calibration curve was constructed by plotting concentration on x-axis and absorbance on y-axis. R^2 value not less than 0.999 was regarded as acceptance criteria **(Table 2, 3).**

Specificity was performed to exclude the possibilities of interference of solvent in the region of maximum absorbance peaks of VENLA and BUPRO. The specificity of the method was tested under the normal conditions and results of the tests proved that the components other than VENLA and BUPRO did not produce the detectable peaks at the maximum absorbance of both the drugs.

Accuracy for the developed methods was determined by recovery studies at three different levels. The pre-analyzed samples were spiked with 75, 100 and 125 % of mixed standard solution. The mixtures were analyzed and the recoveries were determined. The recovery study was carried out in triplicate. The mean recovery of VENLA and BUPRO at each level should not be less than 99.0 % and not more than 102.0 % was considered as the acceptance criteria **(Table 4, 5, 6).**

Precision was studied to find out intra-day and inter-day variations in the test method of VENLA and BUPRO. Intra-day assay precision was found by analysis of standard drug thrice on the same day in different intervals of time. Inter-day assay precision was carried out on three different days and percentage relative standard deviation (% RSD) was calculated. The % RSD should not be more than 2.0 % **(Table 7).**

Sensitivity of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ). The LOD and LOQ of VENLA and BUPRO by proposed methods were determined using calibration standards **(Table 7).**

Ruggedness expresses the variations within the laboratory conditions (different day, different analyst and different instruments.) The ruggedness was performed by analyst 1 and analyst 2 on different instruments on different days **(Table 7).**

RESULTS:

A UV-Spectrophotometric method for simultaneous estimation of VENLA and BUPRO has been developed and validated by using methanol and water as a solvent. The developed method data were presented in **Table 1.** The developed UV-Spectrophotometric method was validated as per ICH guidelines in terms of linearity, specificity, precision, sensitivity, ruggedness and accuracy. The results of validation parameter found to be well within the acceptance limit.

The linearity response of VENLA and BUPRO was observed in the concentration range of 4-24 µg/mL for both the drugs and statistical data such as regression equation and correlation coefficient were found within the well acceptance criteria limit. The results were presented in the **Table 2, 3** and UV spectrum was presented in **Figure 3-6** and standard calibration curve was presented in the **Figure 7 and 8.** The developed method was found to be specific as the solvents used and excipients of tablet formulation showing maximum absorbance of wavelength for VENLA and BUPRO and not interfere in the analysis. This method was found to be accurate as the accuracy results of VENLA and BUPRO showed excellent % recovery values at three different levels. The results of accuracy study were presented in the **Table 4, 5, 6.** The % RSD value of concentration obtained for six replicates of injection of VENLA and BUPRO was found to be less than 2%. Hence developed method was found to be precise. The developed method was found to be sensitive and rugged and results were presented in the **Table 7.** The result of % assay shows that there is no interference of

excipients and no impurities were observed in sample for the developed method. The results of assay were presented in **Table 8.**

Table No. (1): Developed UV method specification

Instrument and Specification	UV-Spectrophotometer Shimadzu 1800
Scanning Range	200 nm to 400 nm
Solvents Used	Methanol and Water
Wavelength of maxima of VENLA	223 nm
Wavelength of maxima of BUPRO	249 nm

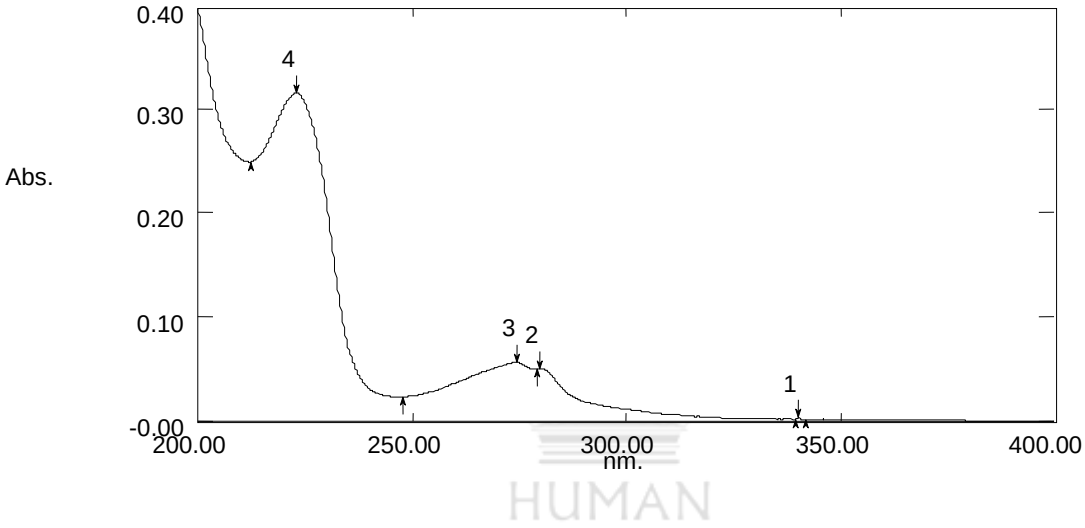


Figure No. (3): UV-Spectrum of Venlafaxine HCl

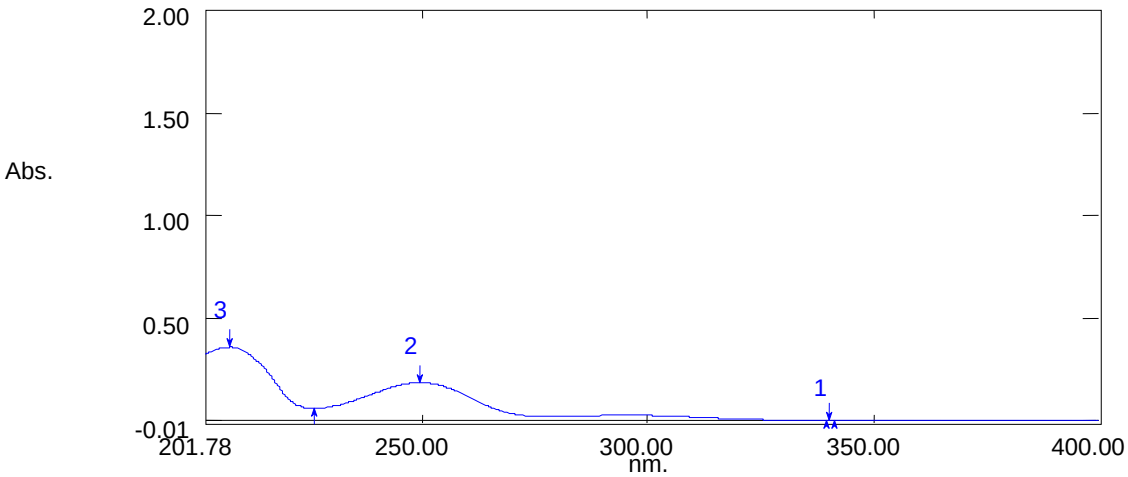


Figure No. (4): UV-Spectrum of Bupropion HCl

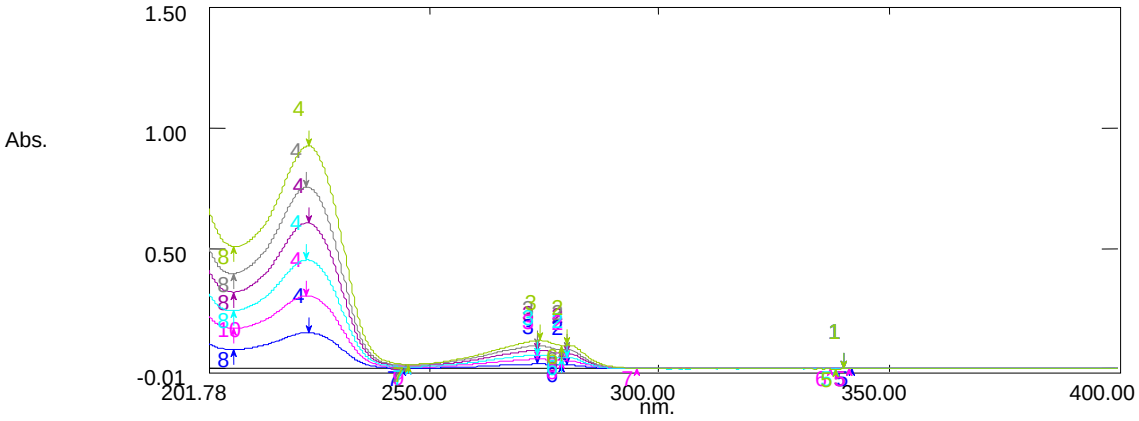


Figure No. (5): Overlay Spectrums of Venlafaxine HCl (4-24 µg/mL)

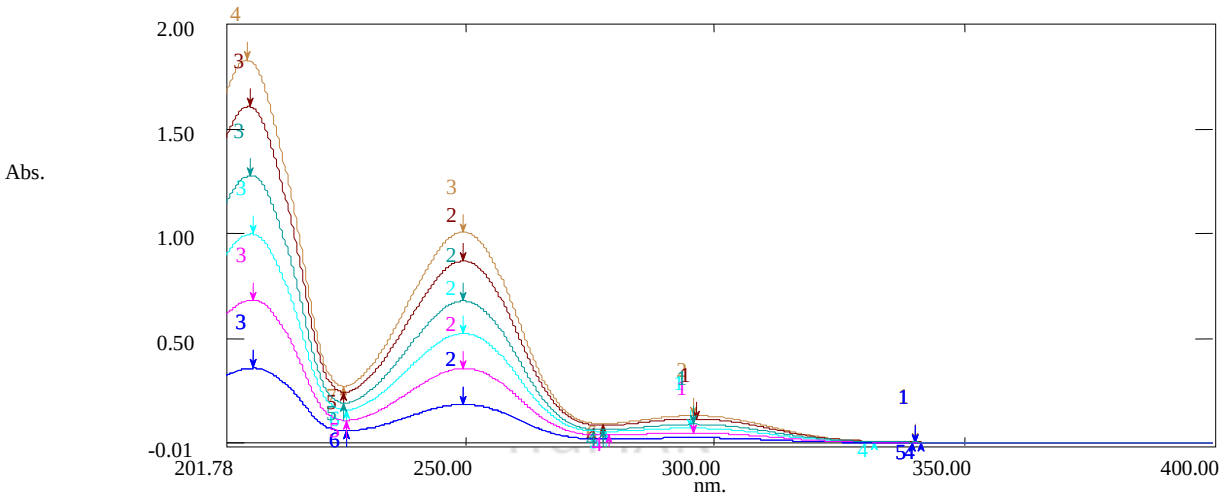


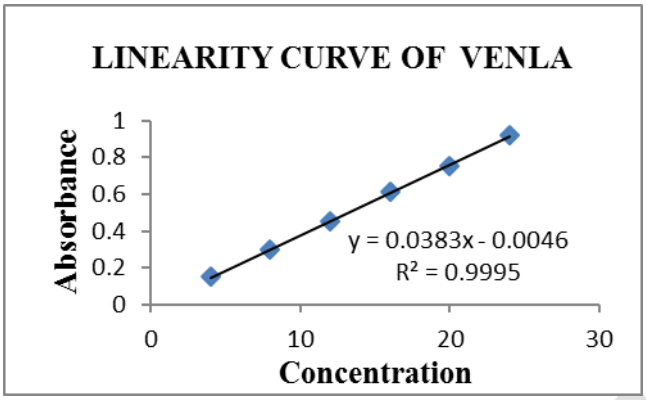
Figure No. (6): Overlay Spectrum of Bupropion HCl (4-24 µg/mL)

Table No. (2): Linearity range data of Venlafaxine HCl and Bupropion HCl

Drugs	VENLA		BUPRO	
Sr. No.	Conc. µg/mL	Abs at 223 nm	Conc. µg/mL	Abs at 249 nm
1	4 µg/mL	0.151	4 µg/mL	0.182
2	8 µg/mL	0.301	8 µg/mL	0.356
3	12 µg/mL	0.452	12 µg/mL	0.522
4	16 µg/mL	0.611	16 µg/mL	0.681
5	20 µg/mL	0.750	20 µg/mL	0.872
6	24 µg/mL	0.921	24 µg/mL	1.012

Table No. (3): Linearity and range report of Venlafaxine HCl and Bupropion HCl

Parameters	VENLA	BUPRO
Linearity range	4-24 µg/mL	4-24 µg/mL
Regression equation	Y= 0.038x – 0.004	Y= 0.041x + 0.018
Correlation coefficient	0.9995	0.9991
Intercept	-0.0046	0.0185
Slope	0.0383	0.0418



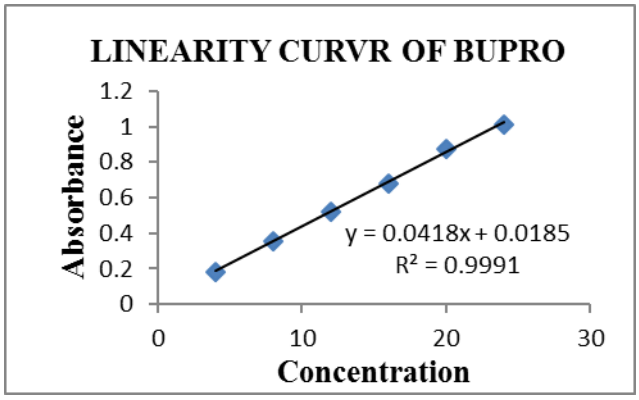


Figure No. (7): Standard calibration curve of Venlafaxine HCl

Figure No. (8): Standard calibration curve Bupropion HCl

Table No. (4): Accuracy study data for Venlafaxine HCl

Level	Replicate	Std conc. (µg/mL)	Sample conc. µg/mL	Conc. found µg/mL	Std recovered µg/mL	% Recovery
75 %	I	2	4	6.000	2.000	100
	II	2	4	6.022	2.022	101.10
	III	2	4	6.000	2.000	100
100 %	I	4	4	8.049	4.049	101.23
	II	4	4	8.000	4.000	100
	III	4	4	8.024	4.024	100.60
125 %	I	6	4	10.000	6.000	100
	II	6	4	10.049	6.049	100.81
	III	6	4	10.098	6.098	101.63

Table No. (5): Accuracy study data of Bupropion HCl

Level	Replicate	Std conc. (µg/mL)	Sample conc. µg/mL	Conc. found µg/mL	Std recovered µg/mL	% Recovery
75 %	I	2	4	6.000	2.000	100
	II	2	4	6.021	2.021	101.05
	III	2	4	6.063	2.063	103.15
100 %	I	4	4	8.000	4.000	100
	II	4	4	8.021	4.021	100.53
	III	4	4	8.064	4.064	101.60
125 %	I	6	4	10.000	6.000	100.37
	II	6	4	10.022	6.022	100
	III	6	4	10.066	6.066	101.1

Table No. (6): Recovery studies Report of Venlafaxine HCl and Bupropion HCl

Levels	Mean % Recovery of VENLA	Mean % Recovery of BUPRO
75 %	100.367	101.4
100 %	100.61	100.71
125 %	100.81	100.49

Table No. (7): Summary of the validation parameter of the proposed method

Parameters	VENLA	BUPRO
Maximum absorbance	223 nm	249 nm
Linearity	4-24 µg/mL	4-24 µg/mL
Correlation coefficient	0.999	0.999
Absorptivity at 223nm	378.507	8.173
Absorptivity at 249nm	146.472	436.382
Precision (% RSD) i. Intra day ii. Inter day	 0.39 0.33	 0.27 0.15
% Recovery	100.59	100.86
LOD	0.42 µg/mL	0.38 µg/mL
LOQ	1.4µg/mL	1.2 µg/mL
Ruggedness	99.3 – 100.7 % (Analyst – 1) 98.0 – 100.3 % (Analyst – 2)	99.2 – 100.3 % (Analyst – 1) 99.2 – 100.5 % (Analyst – 2)
Tablet Assay	101.25 %	100.55 %

Table No. (8): Assay result of Venlafaxine HCl and Bupropion HCl

Drug name	Brand name	Labeled amount	Amount found	% assay
VENLA	VENLOR-XR	150 mg	151.87 mg	101.25 %
BUPRO	BUPRON SR	150 mg	150.82 mg	100.55 %

DISCUSSION

In the present research work, a new UV-Spectrophotometric method for simultaneous estimation of VENLA and BUPRO in bulk and pharmaceutical dosage form was developed and validated. The spectrum of VENLA and BUPRO showed the absorption maxima at 223 nm and 249 nm in methanol and water respectively. The statistical data obtained from the calibration curve of VENLA and BUPRO in solution shows the high level of precision for the developed method, by low value of the coefficient of variation. For the developed method linearity range was observed between 4-24 µg/mL for both the drugs. The plots are clearly showed a straight line passing through the origin. The assay method was validated by precision, accuracy and standard errors by low values of % RSD. The recovery studies prove the excellent accuracy recovery of the method. The ruggedness of the method was studied by using different instrument and analyst. From the above notified values of the recovery study, it can confirm that the method is free from excipients used in the formulations. Based on the obtained results, developed method can be regarded as simple, precise, accurate, and reliable which can be employed for the quality control of VENLA and BUPRO in bulk and pharmaceutical dosage form.

CONCLUSION

The above proposed UV-Spectrophotometric method was found to be simple, precise, specific, sensitive, accurate, reliable and economic for the simultaneous estimation of the VENLA and BUPRO in bulk and pharmaceutical dosage form with the good precision data in % RSD and accuracy with good % recovery of the drugs. The % recovery data shows that the method is free from the excipients used in the formulation and hence it can be used for routine analysis in quality control laboratories.

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