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Development and validation of a new spectrophotometric method for determination of tertiary aliphatic amine derivatives using venlafaxine hydrochloride as the model drug

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ABSTRACT

Development or invention of a new drug(s) definitely necessitates development of analytical method for its estimation in the biological fluids, other solutions and pharmaceutical dosage forms. A new simple, rapid, precise, highly specific and economical spectrophotometric method has been developed and validated for tertiary aliphatic amine derivatives using venlafaxine hydrochloride as the model drug. The method involves formation of the picrate of the amine, extractable into chloroform, which gives a yellow color, but the absorption maximum is located in the ultraviolet range at 352.5nm. Linearity was obtained in the concentration range 10-30µg/mL (r=0.9992). Limit of detection (LOD) and limit of quantitation (LOQ) are 24.69 and 74.80µg/mL respectively. The molar absorptivity and sandell's sensitivity were found to be 1.088×10^5 and 0.003 respectively. The method was optimized and suitably validated following the guidelines for validation on analytical procedures. Validation parameters performed included linearity, accuracy, precision, sensitivity, selectivity and robustness. The method was applied to the commercial formulation of venlafaxine and the results of analysis were statistically validated to determine percent relative standard deviation (% R.S.D.), standard deviation (S.D.), standard error (S.E.) and t-values at 95% confidence level.

KEYWORDS

Spectrophotometry;
Tertiary aliphatic amine;
Venlafaxine;
Colorimetry;
Validation.

INTRODUCTION

Methods of analysis of drugs with tertiary aliphatic amine moiety have not been extensively explored. The scientific novelty of the present work lies in the fact that a new spectrophotometric method has been developed for the analysis of tertiary aliphatic amine using venlafaxine hydrochloride as the model drug. Venlafaxine is chemically described as 1-[2-(Dimethylamino)-1-(4-methoxyphenyl) ethyl] cyclohexanol[1](Figure1).

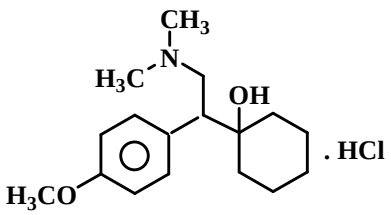

Figure 1: Structure of venlafaxine hydrochloride

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It is a second-generation antidepressant, which selectively inhibits re-uptake of norepinephrine and serotonin, and slightly inhibits re-uptake of dopamine, without significant affinity for muscarinic, histaminergic or α_1- adrenergic receptors[2-3]. A survey of literature reveals that being a novel drug only a few analytical methods have been reported for its determination, in pharmaceutical dosage forms[4-5]. These methods are based on colored chromogen formation. Apart from these a few methods of determination in clinical samples have also been reported, which include high performance liquid chromatography[6-7], liquid chromatography-mass spectrometry[8] and capillary electrophoresis[9]. An extensive and exhaustive revision of the literature revealed that no spectrophotometric method targeting the tertiary aliphatic amine moiety has been reported in literature for the assay of venlafaxine in pharmaceutical dosage forms. The present study hence focuses on the development of an analytical method targeting the tertiary aliphatic amine moiety and also the validation of the developed method. The study was conducted with the objective of developing a simple yet selective and precise method, which can be used for routine analysis as well as formulation screening of tertiary aliphatic amine derivatives.

The method was suitably validated and all optimization parameters were considered.

EXPERIMENTAL

A double beam UV-visible spectrophotometer (Shimadzu, Japan) model UV-1700 (E) 23 OCE with 1cm matched quartz cell was used for experiments. The software version was 1.50, spectral bandwidth was 2 nm and wavelength-scanning speed was 2800nm/min.

Reagents

Dichloromethane and chloroform (Qualigens), picric acid, monosodium phosphate dihydrate and sodium hydroxide (CDH) used were of analytical grade. Purified water was used for the experiments. Reagent A consisted of a mixture of 30g monosodium phosphate dihydrate and 900mg sodium hydroxide in 75mL of water. Reagent B consisted of 0.1% aqueous solution of picric acid.

Standard solutions

Venlafaxine standard solution was prepared by dissolving 25mg (accurately weighed) of venlafaxine standard in 25mL of dichloromethane, yielding stock solution A (1000μg/mL).

General procedure and construction of calibration curves

Aliquots of standard stock solution equivalent to 10-30μg were accurately transferred into a series of 25mL seperatory funnel followed by the addition of 7mL chloroform, 2mL of reagent A and 1mL of reagent B. The funnels were shaken for 1min, the phases allowed to separate and the organic layer collected from each funnel into a series of 10mL volumetric flasks quantitatively. The volume was made upto 10mL with chloroform. The procedure was followed under ambient temperature conditions. The absorbance was measured at 352.5nm against the reagent blank and the calibration curve was plotted.

Application to pharmaceutical formulation

Twenty tablets (Venlor (Cipla) label claim, 75mg) were weighed, crushed, finely powdered and mixed well. Powder equivalent to 25mg of pure venlafaxine was extracted with successive 5ml portions of dichloromethane, filtered and the volume was made upto 25 ml with dichloromethane (1000μg/mL). Aliquots were transferred to 25mL seperatory funnels and the general procedure was then followed as described above. The absorbances were measured accordingly.

RESULT AND DISCUSSION

The method involves formation of the picrate of the amine, extractable into chloroform, which gives a yellow color, but the absorption maximum is located in the ultraviolet range at 352.5nm[10]. The principle involved is ion-pair extraction, using an acid dye as a reagent and a chlorinated solvent as extractant. A detailed predevelopment work was done to devise the optimum reaction conditions. Each parameter was individually optimized. The optimization, validation and statistical analysis are discussed further accordingly.

Optimum reaction conditions

The reaction conditions adopted in the present method were arrived at by optimization study carried in

TABLE 1: Optical characteristics

Parameter	Method
λ_{max}	352.5
Beer's law limits (μg/mL)	10-30
Molar absorptivity (L/mole/cm)*	1.088×10^5
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	0.003
Regression equation (y = mx + c)	
Slope (m)	0.0254
Intercept (c)	0.1622
Correlation coefficient (r)	0.9992
Limit of detection (LOD) (μg/mL)	24.69
Limit of quantitation (LOQ) (μg/mL)	74.80

***Determined from mean absorption coefficient, which is average of six determinations each performed in triplicate**

TABLE 2: Validation data

Study	Method		
	Mean	S.D.	% R.S. D.
Accuracy (% drug recovery)			
80 % level*	98.16	0.46	0.47
100 % level*	99.06	0.81	0.82
120 % level*	98.06	0.61	0.63
Precision	S.E.	S.D.	% R.S. D.
Repeatability#	0.14	0.32	1.30
Interday precision#	-	0.28	1.14
Intraday precision#	-	0.11	0.45
Robustness (stress study)*	-	% Assay	% Degradation
Control sample	-	100.15	-
HCl treated sample	-	92.89	4.69
NaOH treated sample	-	92.77	7.38
H_2O_2 treated sample.	-	89.08	11.07
Heat treated sample.	-	93.91	6.24
UV treated sample.	-	94.47	5.69

***Performed in triplicate; # Denotes average of 3 determinations including six replicate each**

triplicate for each parameter[11-12]. The reactions were conducted at ambient temperature. The colored reaction product formed was scanned in various concentration ranges, to find out the range in which Beer Lambert's law was applicable. A linear correlation was obtained between absorbance and concentration of the drug in a concentration range 10-30μg/mL. Optimum quantity of reagent A and B were found to be 2.0mL and 1.0 mL respectively. The color developed was found to be stable for 24 hours.

Validation of the proposed procedures

To assess the validity of proposed method, ICH norms and commonly accepted guidelines for validation of analytical procedures were adopted[13-18].

Good linearity was manifested as depicted by the value of correlation coefficient (r) as evident from TABLE 1.

In order to obtain precision and accuracy of the proposed method a recovery study was performed (80%, 100% and 120% of the test concentration). To analyze percent drug recovery pure drug was spiked with the common tablet excipients (starch, talc, lactose, HPMC, MCC, magnesium stearate)[19]. The excellent recoveries obtained indicate good accuracy of the proposed method (TABLE 2).

The precision (Interday and Intraday precision) was evaluated by calculating the relative standard deviation of the assay results (TABLE 2). Limits of detection (LOD) and quantitation (LOQ)* and sandell's sensitivity[20] value indicate the sensitivity of the proposed method (TABLE 1).

$$*\text{LOD} = \frac{3.3\times \text{S.D.}}{\text{m}} \quad \text{and} \quad \text{LOQ} = \frac{10\times \text{S.D.}}{\text{m}}$$

where S.D. and m are the standard deviation and the slope of the calibration line.

A synthetic blend consisting of standard drug and commonly used excipients was treated accordingly with the method developed and the calibration curve was plotted. Results obtained were comparable to that for standard drug. Based on these results and the recovery study data it can be concluded that the typical excipients included in the drug formulation do not interfere with the selectivity of the method.

In order to further ascertain the selectivity of the method, different degradation pathways were tried, as the degradation products were not available. The sample solutions were stressed under varying conditions (acidic, alkaline, oxidative, thermolytic and photolytic degradation). The results of degradation study are tabulated in TABLE 2.

Statistical analysis

The proposed method as applied for commercial formulation was statistically analyzed [21-23]. Low values of standard deviation (S.D.), standard error (S.E.) percent, Relative standard deviation (% R.S.D.) and t-values at 95% confidence level indicate the precision of the proposed method (TABLE 3).

The analytical method developed was based on functional group targeting. It was successfully applied to pharmaceutical formulation and was suitably validated. The present work yielded a new spectrophoto-

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TABLE 3: Statistical analysis

Statistical analysis for determination of venlafaxine hydrochloride in tablets								
Sl no.	**Tablet**	**Amount taken(mg)**	**Amount found(mg)***	**S.D.**	**%R.S.D.**	**S.E.**	**'t' calculated**	**'t' theoretical**
1	Venlor	25.0	24.7392	0.32	1.30	0.14	1.8629	2.571

***Average of six determinations. Theoretical 't' values at 95% confidence level for (n-1) degrees of freedom 't' (0.05, 5) = 2.571, n=6**

metric method for analysis of venlafaxine as well as a method for analysis of tertiary aliphatic amine derivatives, which can be applied to the other drugs possessing the same functional group. It is also noteworthy that the present work is less expensive than the published HPLC and capillary electrophoresis methods. The results of validation study and statistical analysis proved that the favorable characteristic of the proposed method lies in its accuracy, selectivity and the ease of performance; suggesting its suitability for the routine analysis and the quality control of pharmaceutical formulations.

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