NEW SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF VENLAFAXINE IN BLUE AND ITS PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Five simple and sensitive methods for the assay of Venlafaxine (VEN) were developed. Method A–C is based on the oxidation of VEN with an excess of oxidant [N–Bromosuccinimide (NBS) in methods A and B or Chloramine–T (CAT) in method C] in acidic medium. The unreacted oxidant is then estimated by using an oxidisable dye [Celestine blue (CB) in method A or Galocyanine (GC) in method C] or p–N–methylaminophenyl sulphate (PMAP)–sulphanilamide (SA) reagent in method B. Method D is based on the formation of coloured molecular complex involving VEN and SNP in the presence of hydroxylamine mono hydrochloride (HA). Method E is based on the formation of coloured coordination complex between cobalt thiocyanate (CTC) and VEN, which can be extracted into nitrobenzene layer from the aqueous solution. Regression analysis of Beer’s law plots showed good correlation in the concentration range of 2–10 μg/mL, 4–20 μg/mL, 2–6 μg/mL, 4–20 μg/mL, 10–30 μg/mL for methods A, B, C, D and E, respectively. The results of analysis have been validated statistically and by recovery studies.

Key words: Venlafaxine, Charge transfer complexation

INTRODUCTION

Venlafaxine (VEN), chemically is (R/S)–1–[2–(dimethylamino)–1–(4–methoxyphenyl) ethyl] cyclohexanol hydrochloride and it is an antidepressant drug. A survey of literature revealed HPLC1–14, LC–MS15–18, GC19, GC–MS20–23 and few spectrophotometric methods have been reported for its determination. The reported spectrophotometric methods possess deficiencies such as low λ_{max} or low sensitivity and the analytically important functional groups of VEN do not seem to have been fully exploited for designing suitable spectrophotometric methods for its determination. Some attempts have been made in the present work in this direction following the five reactions (NBS–CB, method A; NBS–PMAP, method B; CAT –

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GC; method C; SNP–HA; method D; CTC, method E) for the determination of VEN utilizing its structural features. All the methods are applicable to the determination of VEN in bulk form and also in formulations.

**EXPERIMENTAL**

**Instruments:** A Systronic UV–VIS spectrophotometer 117 with 1 cm matched quartz cells was used for all spectral and absorbance measurements. A Systronic digital pH meter 361 was used for pH measurements.

**Reagents:** All reagents were analytical grade and solutions were prepared fresh in double distilled water.

Aqueous solutions of NBS (Loba, 5.618 × 10^{-4} M) CB (Chroma, 5.49 × 10^{-4} M) and HCl (E–Merck, 5 M) were prepared for method A. Aqueous solutions of NBS (Loba, 5.618 × 10^{-3} M), PMAP (Loba, 8.71 × 10^{-3} M) were prepared for method B. Aqueous solutions of CAT (Loba, 7.10 × 10^{-3} M), GC (Chroma, 2.9698 × 10^{-4} M) were prepared for method C. Aqueous solutions of SNP (E. Merck, 1.67 × 10^{-1} M) and NH2OH (Fluka, 7.09 × 10^{-1} M) and Na2CO3 (Loba, 9.43 × 10^{-1} M) were prepared for method D. Aqueous solutions of CTC (2.50 × 10^{-1} M), buffer solution of pH 2.0 and nitrobenzene (Qualigens) for methods E.

**Preparation of drug solutions:** A 1mg/mL stock solution of VEN was prepared by dissolving 100 mg of drug of 100 mL of double distilled water. Working standards 100 µg/mL for method A and E, 200 µg/mL for method B and D, 50 µg/mL for method C. For pharmaceutical formulations, the weighed amount of tablet powder equivalent to 100 mg of VEN was extracted with CHCl3 (3 x 15 mL) and filtered. The combined filtrate was evaporated to dryness and residue was dissolved in 100 mL distilled water. The solution (1mg/mL) was used for the preparation of working sample solution for methods A, B, C and D as described under the preparation of standard drug solution and analysis.

**Method A**

Aliquots of standard VEN solution (0.5 – 2.5 mL, 100.0 µg/mL), 1.25 mL of 5M HCl and 2.5 mL of NBS were delivered into a series of 25.0 mL calibrated tubes and the volume in each tube was made up to 20.0 mL with distilled water. After 10 min, 5.0 mL of CB solution was added and mixed thoroughly. The absorbances were measured after 5 min at 520 nm against distilled water. The blank (omitting drug) and dye (omitting drug and oxidant) solutions were prepared in a similar manner and their absorbances were measured against distilled water. The difference in the decrease in absorbance between test and blank (or test against reagent blank) was observed, which corresponds to the consumed NBS. The drug concentration was computed from its calibration graph.
Method B

Aliquots of standard VEN solution (0.5 – 2.5 mL, 200.0 µg/mL) were transferred into a series of 25.0 mL calibrated tubes. Half mL of 5% AcOH and 2.0 mL of NBS solutions were added to the above solutions and volume in each tube was brought to 10 mL with distilled water and kept aside for 20 min at room temperature. Then 2.0 mL of PMAP solution was added. After 2 min, 2.0 mL of SA solution was added and the volume was made up to the mark with distilled water. The absorbances were measured after 10 min at 520 nm against distilled water. A blank experiment was also carried out omitting the drug. The decrease in the absorbance and in turn, the drug concentration was obtained by subtracting the absorbance of the test solution from the blank. The amount of VEN was computed from its calibration graph.

Method C

To each of 25.0 mL graduated tubes containing standard VEN solution (1.0 – 3.0 mL, 50.0 µg/mL), 1.25 mL of 5M HCl and 2.0 mL of 0.02% CAT were added and the solution was diluted to 20.0 mL with distilled water. After 10 min, 4.0 mL of GC (0.01%) solution was added, mixed thoroughly and the absorbances were measured after 15 min at 540 nm against a reagent blank. A blank was carried out in a similar manner. The decrease in absorbance corresponding to consumed CAT, in turn the drug quantity was obtained by subtracting the absorbance of the blank solution from that of the test solution. The calibration graph was drawn by plotting the decrease in the absorbance of the dye (GC), against amount of the drug. Amount of the drug in any sample was computed from its calibration graph.

Method D

Aliquots of standard drug solution, 200.0 µg/mL, ranging from 0.5 – 2.5 mL were transferred into a series of calibrated tubes and the volume in each tube was brought to 3.0 mL with distilled water. One mL of SNP and 2.0 mL of hydroxylamine (HA) solutions were successively added to each tube and shaken for 2 min. Then 1.0 mL of sodium carbonate solution was added and shaken for 15–25 min. Then contents were diluted to 25.0 mL with distilled water and the absorbance measured after 10 min. at 580 nm (VEN) against the reagent blank. The amount of drug was computed from its calibration graph.

Method E

Aliquots of standard VEN solution (1.0 – 3.0 mL, 100.0 µg/mL) were delivered into a series of calibrated tubes. Two mL of buffer of pH 2.0 and 5.0 mL of CTC solutions were added and the total volume in each tube was adjusted to 15.0 mL with distilled water. These solutions in the tubes were transferred to 125.0 mL separating funnel. To each separating funnel, 10.0 mL of nitrobenzene was added and the contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated nitrobenzene layer was measured after 20 min. at 620 nm against a similar reagent blank. The amount of drug was computed from its calibration curve.
RESULTS AND DISCUSSION

The optimum conditions for each method were established by varying one parameter at a time and keeping the others fixed and observing the effect produced on the absorbance of the coloured species.

The optical characteristics such as Beer’s law limits, molar absorptivity, etc. for each method are given in Table 1. The precision of each method was tested by estimating six replicate samples of the drug within the Beer’s law limits and the results obtained are incorporated in Table 1. Regression analysis was made using the method of least squares to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each method. The accuracy of each method was ascertained by comparing the results by proposed and reference method (UV) statistically (Table 2). This comparison shows that there is no significant difference between the results of proposed methods and those of reference ones. From the similarity of the results, it is evident that during the application of the methods, the additives and excipients that are usually present in tablets do not interfere in the assay of proposed methods.

Table 1. Optical characteristics, precision and accuracy of the proposed methods for VEN

<table>
<thead>
<tr>
<th>Optical Characteristics</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
<th>Method D</th>
<th>Method E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NBS/CB</td>
<td>NBS/PMAPSA</td>
<td>CAT/GC</td>
<td>SNP/HA</td>
<td>CTC</td>
</tr>
<tr>
<td>( \lambda_{\text{max}} , (\text{nm}) )</td>
<td>520</td>
<td>520</td>
<td>540</td>
<td>580</td>
<td>620</td>
</tr>
<tr>
<td>Beer’s Law limits ((\mu\text{g/mL}))</td>
<td>2 – 10</td>
<td>4 – 20</td>
<td>2 – 6</td>
<td>4 – 20</td>
<td>10 – 30</td>
</tr>
<tr>
<td>Molar absorptivity ((L \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}))</td>
<td>2.1590 \times 10^4</td>
<td>8.769 \times 10^3</td>
<td>3.4337 \times 10^4</td>
<td>1.1339 \times 10^4</td>
<td>5.361 \times 10^3</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
<tr>
<td>Sandell’s sensitivity ((\mu g/cm^2/0.001 \text{ absorbance unit}))</td>
<td>0.015</td>
<td>0.036</td>
<td>0.009</td>
<td>0.028</td>
<td>0.059</td>
</tr>
</tbody>
</table>

Regression Equation \((y = a + bc)\)

(i) Slope (b) | 0.06900  | 0.02795  | 0.10840  | 0.03630  | 0.01696  |
(ii) Standard deviation on slope \((S_b)\) | 0.00047  | 0.00013  | 0.00084  | 0.00025  | 0.00008  |
(iii) Intercept (a) | -0.00180 | -0.00060 | 0.00220  | -0.00260 | 0.00220  |
(iv) Standard deviation on intercept \((S_a)\) | 0.00313  | 0.00175  | 0.00357  | 0.00329  | 0.00170  |
(v) Standard error of estimation \((S_e)\) | 0.00299  | 0.00167  | 0.00266  | 0.00314  | 0.00126  |

Relative Standard Deviation * | 0.3047   | 0.3587   | 0.2344   | 0.2070   | 0.2482   |

% of range error (confidence limit)

(i) 0.05 level | 0.255    | 0.300    | 0.196    | 0.173    | 0.208    |
(ii) 0.01 level | 0.377    | 0.444    | 0.290    | 0.256    | 0.307    |

% Error in bulk sample ** | 0.046    | 0.000    | 0.046    | 0.087    | -0.088   |
<table>
<thead>
<tr>
<th>Samples$\textsuperscript{8}</th>
<th>Leveled amount (mg)</th>
<th>Amount found by proposed methods*</th>
<th>Ref. method</th>
<th>% Recovery by proposed methods **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tab I</td>
<td>25</td>
<td>24.96±0.06</td>
<td>24.96±0.05</td>
<td>24.94±0.09</td>
</tr>
<tr>
<td></td>
<td>F=1.33</td>
<td>F=1.11</td>
<td>F=2.92</td>
<td>F=1.06</td>
</tr>
<tr>
<td></td>
<td>T = 1.98</td>
<td>T = 0.99</td>
<td>T = 0.99</td>
<td>T = 0.99</td>
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<tr>
<td>Tab II</td>
<td>25</td>
<td>24.96±0.06</td>
<td>24.92±0.06</td>
<td>24.95±0.09</td>
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<tr>
<td></td>
<td>F=3.09</td>
<td>F=3.06</td>
<td>F=1.40</td>
<td>F=2.69</td>
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<tr>
<td></td>
<td>T = 0.73</td>
<td>T = 0.95</td>
<td>T = 0.38</td>
<td>T = 1.02</td>
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<tr>
<td>Cap I</td>
<td>100</td>
<td>99.2±1.34</td>
<td>98.5±1.57</td>
<td>98.0±1.15</td>
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<tr>
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<td>F=1.84</td>
<td>F=1.77</td>
<td>F=1.05</td>
<td>F=1.76</td>
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<tr>
<td></td>
<td>T = 1.30</td>
<td>T = 1.62</td>
<td>T = 1.15</td>
<td>T = 1.9</td>
</tr>
<tr>
<td>Cap II</td>
<td>100</td>
<td>98.5±1.0</td>
<td>98.0±1.14</td>
<td>98.5±0.69</td>
</tr>
<tr>
<td></td>
<td>F=2.10</td>
<td>F=2.69</td>
<td>F=1.34</td>
<td>F=1.11</td>
</tr>
<tr>
<td></td>
<td>T = 1.3</td>
<td>T = 2.97</td>
<td>t = 2.15</td>
<td>T = 1.90</td>
</tr>
</tbody>
</table>

*Average ± standard deviation of six determinations; the t- and F-values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, t = 2.57, F = 5.05.

**After adding 3 different amounts of the pure labelled to the pharmaceutical formulation, each value is an average of 3 determinations.

$ Different batches from different pharmaceutical companies.
As an additional check of accuracy of the proposed methods, a fixed amount of the drug was added to the preanalysed formulations and recovery experiments were performed. The amount of drug found and the % recovery was calculated in the usual way.

CONCLUSION

The proposed methods are applicable for the assay of drug (VEN) and have the advantage of wider range under Beer’s law limits. The decreasing order of sensitivity and $\lambda_{\text{max}}$ among the proposed methods are $C > A > D > B > E$ and $E > D > C > A = B$, respectively. The proposed methods are simple, selective and can be used in the routine determination of VEN in bulk samples and formulations with reasonable precision and accuracy.

REFERENCES


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