STABILITY INDICATING LIQUID CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF DESVENLAFAXINE IN PHARMACEUTICAL DOSAGE FORM

A simple, sensitive, precise and stability indicating liquid chromatographic method has been developed for the estimation of desvenlafaxine succinate in pharmaceutical dosage form. A Hypersil C-18, 5 µm-column with a mobile phase containing 0.05 M potassium dihydrogen phosphate:methanol (60:40 v/v), pH 7, was used. The flow rate was 1.0 mL/min and effluents were monitored at 226 nm. The retention time of desvenlafaxine was 7.4 min and the method was linear in the range of 0.1-20 µg/mL. Desvenlafaxine stock solution was subjected to acid and alkali hydrolysis, chemical oxidation and dry heat degradation. The drug was found to be susceptible to base hydrolysis and the developed method was found to give good separation between the pure drug and the degraded product. The method was successfully applied to the estimation of desvenlafaxine in tablet dosage forms.

Key words: desvenlafaxine; degradation; reversed phase liquid chromatography; validation.

Desvenlafaxine succinate (DVX) is chemically RS-4-[2-dimethylamino-1-(1-hydroxycyclohexyl)ethyl]-phenol succinate monohydrate. It is a salt form of the major active metabolite of venlafaxine. Desvenlafaxine inhibits serotonergic and noradrenergic reuptake receptors with minimal affinity for muscarinic, cholinergic, histaminergic, and α<sub>1</sub>-adrenergic receptors [1]. Desvenlafaxine lacks monoamine oxidase inhibitory activity. Serotonergic receptors are inhibited approximately 10-fold more than noradrenergic receptors. Based on the in vitro results and the relationship between desvenlafaxine and venlafaxine, desvenlafaxine is classified as a selective serotonin and norepinephrine reuptake inhibitor (SNRI). Approximately 75% of venlafaxine is metabolized into O-desmethylenefaxine or desvenlafaxine by the action of CYP2D6 on parent drug [2]. Desvenlafaxine is not predominately metabolized by the cytochrome P450 (CYP) system and is eliminated primarily by phase II metabolism; as a result, it has lower potential for drug interactions, especially with the CYP 2D6 pathway. It has been approved for the treatment of major depressive disorder (MDD) and has also been studied for the treatment of vasomotor symptoms associated with menopause [3-4]. Preliminary evidence also suggests the clinical usefulness of DVX in the treatment of anxiety symptoms, and painful physical symptoms [5-6].

A literature survey regarding quantitative analysis of the drug revealed that attempts have been made to develop analytical methods for the estimation of DVX in human plasma using HPLC with fluorescence detection [7-9], HPLC with UV detection [10], LC-MS/MS [11-13] and HPLC-ESI/MS [14].

The International Conference on Harmonization (ICH) guidelines [15] require the implementation of stress testing procedures for the identification of degradation products that are potentially occurring in drug substances which can help to understand the possible degradation pathway for the drug. The apparent lack of a method for the estimation of desvenlafaxine in pharmaceutical dosage form prompted us to develop an accurate, specific and sensitive liquid chromatographic method.

EXPERIMENTAL

Apparatus

The liquid chromatographic system consisted of a Perkin Elmer HPLC model (200 series) containing
The calibration curve (n = 5) was constructed by plotting peak area versus concentration of the drug and regression equations were computed.

Validation

The method was validated for accuracy, precision, specificity, detection limit, quantitation limit and robustness as per ICH guideline [15].

Accuracy. The accuracy of the method was determined by calculating recoveries of DVX by method of standard additions. Known amounts of DVX (0, 4, 7.5 and 12 µg/mL) were added to a pre-quantified sample (7.5 µg/mL) and the amounts of DVX were estimated by measuring the peak area and by fitting these values to the straight-line equation of calibration curve.

Precision. The instrumental precision was evaluated by injecting the solution containing DVX (10 µg/mL) six times repeatedly and measuring the peak area. The results are reported in terms of relative standard deviation. The intra-day and inter-day precision study of DVX was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days (first, second and third day) for 3 different concentrations of DVX (0.1, 5 and 20 µg/mL) and the results are reported in terms of relative standard deviation (RSD).

Specificity. The specificity was estimated by spiking commonly used excipients (starch, talc and magnesium stearate) into a pre-weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

Limit of detection and quantification. The detection limit is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using following equation as per ICH guidelines.

\[
\text{LOD} = 3.3\sigma/S \quad \text{and} \quad \text{LOQ} = 10\sigma/S, \quad \text{where} \ \sigma \ \text{is the standard deviation of y-intercepts of regression lines and} \ S \ \text{is the slope of the calibration curve.}
\]

Robustness. Robustness of the method was studied by deliberately changing the experimental conditions like flow rate, percentage of organic phase and also by observing the stability of the sample solution at 25±2 °C after 24 h.

Forced degradation study

Stress degradation study using acid and alkali hydrolysis, chemical oxidation and dry heat degradation was carried out and interference of the degradation products was investigated. DVX was weighed (25 mg each) and transferred to a 25 mL volumetric flask and diluted up to the mark with mobile phase. This stock solution was used for forced degradation studies.

a) Base hydrolysis. Forced degradation in basic media was performed by taking 2.5 mL stock solution of DVX in 25 mL volumetric flasks and 5 mL of 1 M...
NaOH was added. The solution was heated in a water bath at 70 °C for 1 h and allowed to cool to room temperature. Solution was neutralized with 1 M HCl using pH meter and suitably diluted with mobile phase to obtain final concentration of 10 µg/mL of DVX.

b) Acid hydrolysis. Forced degradation in acidic media was performed by taking 2.5 ml of stock solution of DVX in 25 mL volumetric flasks and 5 ml of 1 M HCl was added. The flask was heated in a water bath at 70 °C for 1 h and allowed to cool to room temperature. Solution was neutralized with 1 M NaOH using pH meter and suitably diluted with mobile phase to obtain final concentration of 10 µg/mL of DVX.

c) Oxidative stress degradation. To perform oxidative stress degradation, 2.5 mL stock solution of DVX was taken in a 25 mL volumetric flask and 5 mL of 3% hydrogen peroxide was added. The flask was heated in a water bath at 70 °C for 1 h and allowed to cool to room temperature. Solution was appropriately diluted with mobile phase to obtain final concentration of 10 µg/mL of DVX.

d) Dry heat degradation. To study dry heat degradation, solid drug was exposed in oven at 80 °C for 2 h. The solid was allowed to cool and 25 mg of DVX was weighed, transferred to volumetric flask (25 mL) and dissolved in few mL of methanol. Volume was made up to the mark with the methanol. Appropriate aliquot was taken from above solution and diluted with mobile phase to obtain final concentration of 10 µg/mL of DVX.

All the reaction solutions were injected in the liquid chromatographic system and chromatograms were recorded.

Analysis of marketed formulations

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 50 mg of DVX was accurately weighed and transferred to a 50 mL volumetric flask. A few mL (20 mL) of mobile phase was added to the above flask and sonicated for 5 min. The solution was filtered using Whatman filter paper No. 1 in another 50 mL volumetric flask and volume was diluted to the mark with the mobile phase.

Appropriate volume of the aliquot was transferred to a 25mL volumetric flask and the volume was made up to the mark with mobile phase to obtain 7.5 µg/mL of DVX. The solution was injected at above chromatographic conditions and peak areas were measured. The quantification was carried out by keeping these values to the straight line equation of calibration curve.

RESULTS AND DISCUSSION

Optimization of mobile phase

Optimization of mobile phase was performed to obtain a well resolved peak for active drug ingredient (DVX) and degradation products produced under stressed conditions. Various mixtures containing aqueous buffer-methanol were tried as mobile phases.

Different mobile phases tried were methanol:0.025 M potassium dihydrogen phosphate (80:20 v/v) which gave peak for DVX at 2.95 min with unsatisfactory peak resolution, methanol:0.025 M potassium dihydrogen phosphate (80:20 v/v), pH 6.8, which gave peak for DVX at 3.45 min and methanol: 0.05 M potassium dihydrogen phosphate (60:40 v/v), pH 8, which gave peak for DVX at 3.93 min.

The mobile phase consisting of 0.05 M potassium dihydrogen phosphate:methanol (60:40, v/v), pH 7, was selected which gave sharp peak for DVX (Figure 1). The flow rate was maintained at 1.0 mL/min. The retention times for DVX was 7.4 min. Ultraviolet spectra of DVX showed that the drug absorbed appreciably at 226 nm, so the same was selected as the detection wavelength during chromatographic studies.

Validation

The developed method was validated as per ICH guideline. The calibration curve (n = 5) was found to be linear over the range of 0.1-20 µg/mL for DVX. The data of regression analysis of the calibration curves are shown in Table 1. The accuracy of the method was determined by calculating recoveries of DVX by method of standard additions. The recoveries were found to be 95.81-102.2%. The high values indicate that the method is accurate. Instrument precision was determined by performing injection repeatability test and the RSD value for DVX were found to be 1.2%. The intra-day and inter-day precision studies were carried out. For the intra-day study RSD values were found to be 0.8-1.3% for DVX and for inter-day precision study RSD values were found to be 1.2-1.6% for DVX. The low RSD values indicate that the method is precise. The detection limit for DVX was 0.03 µg/mL, while quantitation limit was 0.1 µg/mL, respectively. The above data shows that a nanogram quantity of drug can be accurately and precisely determined. The validation parameters are summarized in Table 2.

Robustness of the method was studied by changing the flow rate of the mobile phase from 1mL/min to 0.8 and 1.2 mL/min. Using 1.2 mL/min flow rate, retention time for DVX was observed to be 6.05 min and with 0.8 mL/min flow rate retention times for DVX was
observed to be 9.08 min. When mobile phase composition was changed to 0.05 M potassium dihydrogen phosphate:methanol (55:45 v/v) by increasing percentage of methanol, the retention time for DVX was observed to be 5.72 min. The solution stability study revealed that DVX solution was stable for 24 h without detectable degradation and the percentage recovery of was found to be more than 98%.

Table 1. Regression analysis of calibration curve

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DVX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range, μg/mL</td>
<td>0.1-20</td>
</tr>
<tr>
<td>Slope</td>
<td>29470.83</td>
</tr>
<tr>
<td>Standard deviation of slope</td>
<td>367.5657</td>
</tr>
<tr>
<td>Intercept</td>
<td>6313.833</td>
</tr>
<tr>
<td>Standard deviation of intercept</td>
<td>303.463</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.998</td>
</tr>
</tbody>
</table>

Forced degradation study

The chromatograms of base degraded sample showed degradation product peaks at retention time (RT) 2.45, 3.51, 4.01 and 6.13 min (Figure 2). The degradation product peaks were well resolved from drug peak ($R > 2$). The chromatogram of acid degraded samples showed no degradation products peaks. DVX was found to be stable to acid hydrolysis at room temperature and at 70 °C (Figure 3). Oxidative stress degradation sample showed degradation peak at retention time 4.12 and 4.50 min. Peaks around 0.2-1.5 min in the chromatogram is because of the impurities present in the hydrogen peroxide (Figure 4). Dry heat degradation study revealed that there is no degradation peak for DVX (Figure 5). The drug was found to be stable.

Table 2. Summary of validation and system suitability parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DVX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time, min</td>
<td>7.4</td>
</tr>
<tr>
<td>Asymmetry</td>
<td>1.3</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>11469</td>
</tr>
<tr>
<td>Detection limit, μg/mL</td>
<td>0.03</td>
</tr>
<tr>
<td>Quantitation limit, μg/mL</td>
<td>0.1</td>
</tr>
<tr>
<td>Accuracy, %</td>
<td>95.81-102.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Precision (RSD / %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-day ($n = 3$)</td>
<td>0.8-1.3</td>
</tr>
<tr>
<td>Inter-day ($n = 3$)</td>
<td>1.2-1.6</td>
</tr>
<tr>
<td>Instrument precision</td>
<td>1.2</td>
</tr>
</tbody>
</table>

The forced degradation study thereby indicated that DVX was found to be susceptible to base hydrolysis and oxidative stress degradation while it was stable to acid hydrolysis and dry heat degradation study (Table 3). The degradation product peaks were well resolved from drug peak with a resolution of more than 2.
Figure 2. Chromatogram of base treated DVX (10 μg/mL) at 70 °C for 1 h.

Figure 3. Chromatogram of acid treated DVX (10 μg/mL) at 70 °C for 1 h.
Figure 4. Chromatogram of hydrogen peroxide (3%) treated DVX (10 μg/mL) at 70 °C for 1 h.

Figure 5. Chromatogram of DVX (10 μg/mL) after dry heat exposure at 80 °C for 2 h.
Table 3. Forced degradation study of DVX for the proposed LC method

<table>
<thead>
<tr>
<th>Medium</th>
<th>Conditions</th>
<th>Recovery, % (DVX)</th>
<th>Retention times of degradation products, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base, 1 M NaOH</td>
<td>70 °C for 1 h</td>
<td>54.94</td>
<td>2.45, 3.51, 4.01 and 6.13</td>
</tr>
<tr>
<td>Acid, 1 M HCl</td>
<td>70 °C for 1 h</td>
<td>98.45</td>
<td>-</td>
</tr>
<tr>
<td>3% Hydrogen peroxide</td>
<td>70 °C for 1 h</td>
<td>86.58</td>
<td>4.12 and 4.50</td>
</tr>
<tr>
<td>Dry heat</td>
<td>80 °C for 2 h</td>
<td>97.25</td>
<td>-</td>
</tr>
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</table>

Analysis of marketed formulations

The proposed method was applied to the determination of DVX in tablet dosage form. The assay results obtained were 98.88±0.45 (n = 3) which was comparable with the corresponding labeled amount.

CONCLUSION

The proposed study describes a stability indicating liquid chromatographic method for the estimation of DVX in pharmaceutical dosage form. The method was validated and found to be simple, sensitive, accurate and precise. The method was successfully used for determination of drug in its pharmaceutical formulation. Also, the above results indicate the suitability of the method for acid, base, dry heat and oxidative degradation study of drug. As the method separates the drugs from its degradation products, it can be used for analysis of stability samples of drug. In future, isolation and characterization of degradation product can be carried out and pharmacological effect of isolated degradation product can be evaluated.

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REFERENCES

ODREĐIVANJE SADRŽAJA I STABILNOSTI DESVENLAFAKSINA U FARMACEUTSKIM PREPARATIMA METODOM TEČNE HROMATOGRAFIJE

Desvenlafaksin je jedna od novijih psihoterapijskih lekarica. Racionalna kombinacija prema učinkovitosti i sigurnosti je često korisna. U ovom radu je razvijena tečna hromatografska metoda za određivanje sadržaja i stabilnosti desvenlafaksina u farmaceutskim preparatima.

Metoda je testirana na mobilnoj fazi 0,05 M kalijum-dihidrog-fosfat:metanol (60:40 v/v), pH 7. Protok je bio 1,0 ml/min, a eflekti su proučeni na 226 nm. Retenciono vreme desvenlafaksina je 7,4 min, a linearnost važi u opsegu koncentracije 0,1-20 µg/ml.

Sadržaj desvenlafaksina je podvrgnut kiselinskoj i baznoj hidrolizi, hemijskoj oksidaciji i suvoj termičkoj degradaciji. Utvrđeno je da je lek osetljiv na baznu hidrolizu i da razvijena metoda dobro razdvaja čist lek od degradacionih proizvoda.

Ova metoda je uspješno primijenjena za određivanje desvenlafaksina u tabletama.

Ključne reči: Desvenlafaksin, degradacija, tečna hromatografija na obrnutim fazama, validacija.