STABILITY INDICATING UV-SPECTROPHOTOMETRIC METHOD FOR SECNIDAZOLE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

Secnidazole is a nitroimidazole anti-infective agent which has chemical structure susceptible to degradation. It shows activity against anaerobic micro-organisms and appears effective in the treatment of amoebiasis, giardiasis, trichomoniasis and bacterial vaginosis. In this work forced degradation studies of secnidazole were carried out by developed & validated spectroscopic method. A simple, accurate, precise and economical spectroscopic method have been developed and validated for the determination of secnidazole in their bulk and pharmaceutical formulation at 320 λ max in double distilled water by using UV-spectrophotometer. Beer’s law was obeyed in the concentration range of 4-12 ppm. Good accuracy (90.50%), precision (%RSD 1.50), LOD (0.33µg/ml), LOQ (1.003µg/ml) and linearity (0.993) were obtained. The stress degradation study was carried out according to the ICH requirements (Q1A (R2) and Q1B) include acid, alkali, H₂O₂, and photolytic degradation. The result showed that the drug degrades more under alkaline (37.76%) and acidic (21.33%) condition than other stress conditions. The proposed method and stress degradation study can be used for routine quality control analysis of secnidazole in bulk and pharmaceutical formulation.
INTRODUCTION:
According to an FDA guidance document, a stability-indicating method is a validated quantitative analytical procedure that can detect the changes with time in the properties of the drug substance and drug product [1]. Stability testing is an important part of drug development process; it provides evidence on how the quality of drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light [2]. The objective of the stress testing is to anticipate the behaviour of the drug substance when using it as a drug product [3]. Stress testing of the drug substance can help to establish the degradation pathways and the intrinsic stability of the molecule and developed stability-indicating analytical methods. A stability-indicating method accurately measures the active ingredients without interference from degradation products, process impurities, excipients or other potential impurities. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved [4].

Fig. 1 Structure of secnidazole
Secnidazole [1-(2-methenol-5-nitro-1-H-imidazole-1-yl)] (Fig. 1) is rapidly and completely absorbed after oral administration and has a longer terminal elimination half-life (approximately 17 to 29 hours) than commonly used drugs in this class. In patients with intestinal amoebiasis or giardiasis, clinical or parasistological cure rates of 80 to 100% are achieved after treatment with a single dose of secnidazole 2 g (30 mg/kg in children). Patients with hepatic amoebiasis appears to respond well to 5 to 7 day therapy with secnidazole,. Clinical improvement and/or microbiological evidence of cure were attained in approximately 59 to 96% of patients. In the clinical trials reviewed, secnidazole was well tolerated; most adverse events were gastrointestinal in nature and did not require treatment intervention or withdrawal from therapy. Secnidazole is official in BP and EP [5-6].

In summary, available evidence suggests that secnidazole is as efficacious as other 5-nitroimidazole drugs in the treatment of protozoal infections and bacterial vaginosis. The convenience and ease of administration associated with single-dose therapy, combined with a good tolerability profile, make secnidazole a suitable option to other single-dose treatments and an attractive alternative to multiple dosage regimens with other drugs in this class [7].
Literature survey reveals several spectroscopic, HPLC and HPTLC methods for the estimation of secnidazole was reported. However there was no method reported for stress degradation of secnidazole by UV spectrophotometry.

MATERIALS AND METHODS

Material

Pure samples: Secnidazole was kindly supplied by Cipla Pharmaceuticals Ltd., Mumbai, Maharashtra, India.

Marketed Formulation

SECNIL FORTE™ was purchased from an open market for this study, which contains secnidazole 1 gm.

Instrumentation and chemicals

All absorbance measurements were done with Jasco V-630; double beam UV-Spectrophotometer (Japan) with 10 mm matched quartz cell and borosil glass wares were used for the study. The software is SPECTRA MANAGER. All weighing were done on contech CB 50 electronic balance, Clean Ultrasonicator (Spectralab UC 40) was also used during the analysis. All the chemicals and reagents used were of analytical grade (AR) procured from Thomas Baker (chemicals) Pvt. Ltd.

Selection of media

The criteria for selection of media is solubility and stability i.e. drug should be soluble as well as stable for sufficient time in selected media. Secnidazole was freely soluble in distilled water, methanol and acetonitrile. It was freely soluble in distilled water and was considerably stable. Hence double distilled water was selected as solvent.

Preparation of Standard Stock Solutions

10 mg of secnidazole working standard was weighed accurately and transferred to a 10 ml volumetric flask. Solution was sonicated and diluted up to the mark with double distilled water.

Preparation of Working Standard Solutions

The prepared stock solution was further diluted with double distilled water to get working standard solutions of 10 ppm of the drug. To construct Beer’s law plot for pure drug, different aliquots of the drug were taken and diluted to 10 ml with double distilled water.

Scanning and Determination of Maximum Wavelength (λ\text{max})

In order to ascertain the wavelength of maximum absorption (λ\text{max}) of the drug, different solutions of the drugs (4 μg/ml, 6 μg/ml, 8 μg/ml, 10 μg/ml, 12 μg/ml) in double distilled water was scanned using spectrophotometer within the wavelength region of 200–400 nm against double distilled water as blank. The absorption curve showed characteristics absorption at 320 nm for secnidazole. (Fig. 2).
METHOD VALIDATION

Linearity
The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of secnidazole. Beer-Lambert’s law was obeyed in the concentration range of 4 to 12 ppm.

Precision
The precision was studied in terms of changes in absorbance of drug solution on the same day and inter-day changes on two different days. The intra-day and inter-day variations were calculated in terms percentage relative standard deviation (%RSD).

Accuracy (Recovery)
Recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%) of the bulk sample of secnidazole to the previously analyzed solution of marketed formulation (SECNIL FORTE).

Robustness
The procedure was carried out by changing the (λ max) ± 2nm.

Limit of detection and Limit of quantification
Limit of detection (LOD) and Limit of quantification (LOQ) of secnidazole was calculated by using equation given in the ICH Q (2) B guidelines.

Determination of drug in their dosage forms: (Assay)
Twenty tablets were accurately weighed and a quantity of tablet powder equivalent to 1 mg of secnidazole was weighed and dissolved in 10 ml double distilled water. The solution was then filtered and diluted further to obtain final concentration 10 µg/ml. The sample solution was analyzed and the % drug content was determined from the absorbance using the specific absorbance (A1%1cm).
STRESS DEGRADATION STUDY

Acid degradation
Solution for acid degradation study was prepared in 0.01 N HCl, refluxed for 5 h at 70°C in water bath. The specific amount of solution (0.1 ml) was withdrawn after every hour (0-5 h), diluted up to 10 ml with double distilled water and absorbance was measured by scanning the prepared solution.

Alkali degradation
Solution for alkali degradation was prepared in 0.01 N NaOH, added few drops of methanol to make the drug soluble. The solution was refluxed for 5 h at 70°C in water bath, 0.1 ml solution was withdrawn after every hour (0-5 h), diluted up to 10 ml with double distilled water and absorbance was measured by scanning the prepared solution.

Oxidation with $H_2O_2$
Solution for oxidation degradation study was prepared in 3% $H_2O_2$, added few drops of methanol to make the drug soluble. The solution was placed in cupboard for 5 h, 0.1 ml solution was withdrawn after every hour (0-5 h), diluted up to 10 ml with double distilled water and absorbance was measured by scanning the prepared solution.

Photolytic degradation
Solution for photolytic degradation study was kept in direct exposure of sunlight. Initially at 0 hr take 0.1 ml of this solution and volume was made up to 10 ml with double distilled water and then withdrawing the specific amount of drug at every hour (0-5 h). Absorbance was measured by scanning the prepared solution.

RESULTS AND DISCUSSION:

Linearity:
The linearity of measurement was evaluated by analyzing different concentration of the standard solutions of secnidazole. The Beer-Lambert’s concentration range was found to be 4-12 μg/ml. The correlation coefficient ($r^2$) was found to be 0.993. This indicates that method has good linearity range (Fig. 3).

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.2386</td>
</tr>
<tr>
<td>6</td>
<td>0.3594</td>
</tr>
<tr>
<td>8</td>
<td>0.4845</td>
</tr>
<tr>
<td>10</td>
<td>0.6433</td>
</tr>
<tr>
<td>12</td>
<td>0.7345</td>
</tr>
</tbody>
</table>

Table: 1 Linearity of secnidazole

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Precision
The precision was carried out by taking five replicates of the 3 different concentrations (4, 8 and 12 ppm) which was taken Intra-day and Inter-day and absorbance was recorded, from which the standard deviation (SD) and %Relative Standard Deviation (%RSD) is calculated. The % RSD observed was less than 2 (within the prescribed limits as per the official guidelines). *(Table 2)*

**Table: 2 Intra-day & Inter-day Precision**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Relative Standard Deviation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intra-Day</td>
</tr>
<tr>
<td>4PPM</td>
<td>1.86</td>
</tr>
<tr>
<td>8PPM</td>
<td>1.28</td>
</tr>
<tr>
<td>12PPM</td>
<td>1.61</td>
</tr>
</tbody>
</table>

Accuracy (Recovery)
The recovery was conducted at three different labels 80%, 100% & 120% with the tablet formulation. The recovery of sample is 100.45, 86.18 and 84.88. *(Table 3)*

**Table: 3 Accuracy of developed method**

<table>
<thead>
<tr>
<th>Level of % Recovery</th>
<th>% Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>100.45</td>
</tr>
<tr>
<td>100</td>
<td>86.18</td>
</tr>
<tr>
<td>120</td>
<td>84.88</td>
</tr>
</tbody>
</table>
Robustness

The robustness was performed by changing absorbance maxima by ±2 nm; method indicates very less variation in the procedure (Table 4).

Table: 4 Robustness of developed method

<table>
<thead>
<tr>
<th>10 ppm</th>
<th>318</th>
<th>320</th>
<th>322</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.6154</td>
<td>0.6170</td>
<td>0.6151</td>
</tr>
</tbody>
</table>

Limit of detection and Limit of quantification

LOD and LOQ were determined on the basis of standard deviation and calibration curve. Formula for LOD and LOQ is given in ICH Q (2) B guideline.

LOD = 3.3 × S.D./S
LOQ = 10 × S.D./S

Where, S.D = standard deviation & S = slope of calibration curve.

Table: 5 LOD and LOQ

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3312 µg/ml</td>
<td>1.0039 µg/ml</td>
</tr>
</tbody>
</table>

Results for Stability Study

Table 6 represents the result of stability studies for secnidazole. Result showed that the absorbance for secnidazole in all stressed conditions was decreased from 0-5 h. Therefore, the drug secnidazole undergoes degradation in all stressed conditions. Secnidazole showed more degradation in alkaline medium (37.7%) as compared to acid (21.3%), oxidative (15.6%), and photolytic (12.21%) stress condition. Based on the results obtained, it was found that the proposed stability indicating method can be employed for routine quality control and stability study of secnidazole in its pharmaceutical dosage form.

Table: 6 Summaries of Stress Degradations Results

<table>
<thead>
<tr>
<th>Stress Condition</th>
<th>Time (Hours)</th>
<th>% Assay of Degraded product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic Degradation</td>
<td>5 hrs.</td>
<td>21.33%</td>
</tr>
<tr>
<td>Alkaline Degradation</td>
<td>5 hrs.</td>
<td>37.76%</td>
</tr>
<tr>
<td>Oxidative Degradation</td>
<td>5 hrs.</td>
<td>15.6 %</td>
</tr>
<tr>
<td>Photolytic Degradation</td>
<td>5 hrs.</td>
<td>12.21%</td>
</tr>
</tbody>
</table>
CONCLUSION
A simple, precise, rapid and accurate stability indicating UV method for determination of secnidazole from pure and its dosage forms has been developed and validated. Recoveries in formulation were in good agreement with their respective label claims. The proposed methods can be used for the routine determination of secnidazole in bulk and pharmaceutical dosage forms. The proposed UV-spectrophotometric method has been evaluated over the linearity, accuracy, precision, robustness, LOD and LOQ. The proposed method was found to be convenient and effective for the quality control and stability studies of secnidazole. The results obtained from the stress testing showed that the drug substance is particularly unstable under alkaline condition than acidic, oxidation and photolytic condition. Therefore, care should be taken in the manufacturing process and during storage of this product in order to avoid degradation because, if the drug is degraded could result in diminution of the therapeutic activity and safety.

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REFERENCES