Research Article

METHOD DEVELOPMENT OF POTENT PYRIMIDINE DERIVATIVE HAVING ANTICANCER, ANTIOXIDANT AND ANTIFUNGAL ACTIVITY

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ABSTRACT

A rapid and sensitive U.V. visible spectroscopic method was developed for the estimation of pyrimidine 3,4-dihydro-1-(tetrahydro-3,4-dihydroxy-5-(hydroxymethyl) furan-2-yl) -6-(4-nitrophenyl)-4-phenylpyrimidin-2(1H)-one (6N) in bulk form. Pyrimidine derivative was monitored at 314 nm with U.V. detection and there is no interference of diluents at 314 nm. The method was found to be linear in the range of 10 - 50 mcg/ml. The accuracy and precision were determined and validated statistically. The method was validated as per International conference on harmonization (ICH) guideline. The results showed that proposed method is suitable for the accurate, precise and rapid determination of pyrimidine derivative.

Keywords: Pyrimidine, U.V. Spectroscopy, Calibration, Validation.

INTRODUCTION

Nitrogen containing heterocyclic ring such as pyrimidine is promising structural moiety for drug design. Pyrimidine derivative form a component in various useful drugs and are associated with many biological and therapeutically activities. Condensed pyrimidine have been reported as antimicrobial1-3 anti-inflammatory,4,5 analgesic6,7 anticancer,8-10 anti-HIV,11 antitubercular, antimalarial, diuretic and cardiovascular disease.12

The present work is synthesis, biological evaluation and validation of novel pyrimidine derivatives. Research worker have synthesized 40 pyrimidine derivatives. Among them 6N exhibited maximum antimicrobial, anti-oxidant and anticancer activity. Hence validation studied was done on 6N.

6N is chemically 3,4-dihydro-1-(tetrahydro-3,4-dihydroxy-5-(hydroxymethyl) furan-2-yl) -6-(4-nitrophenyl)-4-phenylpyrimidin-2(1H)-one. It is a yellow crystalline powder with molecular formula is C21H21N3O7 and molecular weight is 427. It is a potent antifungal, antioxidant and anticancer agent among the entire synthesized derivative.

Figure 1: Chemical structure of 6N

Hence the aim of present investigation is to develop simpler, rapid and cost effective analytical method for the
determination of pyrimidine derivative (6N) in bulk dosage form suitable for routine quality control analysis.

Method validation is the process used to confirm that analytical procedure employed for specific test is suitable for its intended use. It is an integral part of any good analytical practice. Methods need to be validated or revalidated.

(a) Before their introduction into routine use.
(b) Whenever the conditions change for which the method has been validated, e.g., instrument with different characteristics.
(c) Whenever the method is changed, and the change is outside the original scope of the method.

**Advantage of analytical method validation**

(a) The biggest advantage of method validation is that it builds degree of confidence, not only for the developer but also to the user.
(b) Although the validation exercise may appear costly & time consuming, it results inexpensive, eliminates frustrating repetitions and leads to better time management in the end.
(c) Minor changes in the conditions such as reagent supplier and grade, analytical set up are unavoidable due to obvious reasons but the method validation absorb shock of such conditions and pays for more than invested in the process.

**EXPERIMENTAL**

2 Experimental

2.1 Chemical and Reagent

6N was synthesized by research worker and then validated. Methanol were used throughout spectrophotometric method development and validation.

2.2 Instrumentation

U.V. Spectrophotometric method was performed on double beam U.V. Visible spectrophotometer (shimadzu model 1800) having two matched quartz cells with 1 cm light path.

2.3 Determination of maximum wavelength (\(\lambda_{max}\))

About 50 mg of 6N was weighed accurately and transferred into 50 ml volumetric flask and dissolved in 25 ml of methanol and made up to the volume with same solvent mixture to give a standard concentration of 1000 µg/ml. By that solution prepared concentration of 20 mcg/ml. This solution was scanned against blank over the entire U.V. Visible wavelength of 200-800. Based on the spectrum a \(\lambda_{max}\) of 314 nm was selected for further analysis.

2.4 Standard preparation (Methodology)

About 50 mg of 6N was weighed accurately and transferred into 50 ml volumetric flask and dissolved in 25 ml of methanol and made up to the volume with same solvent mixture to give a standard concentration of 1000 µg/ml. prepared 10, 20, 30, 40 & 50 mcg/ml. Absorbance of these standard were measured at \(\lambda_{max}\) of 314 nm. Standard curve was drawn by plotting concentrations Vs absorbance.

2.5 Sample preparation:

About 50 mg of 6N was weighed accurately and transferred into 50 ml volumetric flask and dissolved in 25 ml of methanol and made up to the volume with same solvent mixture to give a standard concentration of 1000 µg/ml. filter the solution and to get a standard concentration of 20 µg/ml. The absorbance of the resulting solution was measured at 314 nm. The actual concentration of drug in the sample was determined from the standard curve.

3. Validation

The methods were validated with respect to Linearity, Accuracy, Precision, Specificity, Ruggedness, Robustness, Limit of Detection (LOD) and Limit of Quantitation (LOQ).

3.1 Linearity:

The Linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of analyte in the samples within a given range.

For assay determination the concentration of 6N, is 20 mcg/ml. So the working range of analyte was set between 10, 15, 20, 25 to 50 mcg/ml to show the linearity of the curve obtained. Linearity standard solutions 10, 15, 20, 25 and 50 mcg/ml corresponding to approximately 10, 15, 20, 25 to 50 % of the test concentration where prepared as per Methodology. The observations and calibration curve is shown in table 1 and fig 2.

3.2 Accuracy (By Recovery test)

Accuracy of method is by shown by recovery study and spiking working standard in the placebo at levels 80, 100 and 120 % of the working standard. Recovery study was performed by spiking in 6N to the placebo at levels 80, 100 and 120% of working standard. The samples were
**Table 1:** Linearity table of 6N in working standard

<table>
<thead>
<tr>
<th>S. No</th>
<th>Approx concentration on mcg/ml</th>
<th>Absorbance at 314 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>10</td>
<td>0.288</td>
</tr>
<tr>
<td>2.</td>
<td>15</td>
<td>0.559</td>
</tr>
<tr>
<td>3.</td>
<td>20</td>
<td>0.895</td>
</tr>
<tr>
<td>4.</td>
<td>25</td>
<td>1.298</td>
</tr>
<tr>
<td>5.</td>
<td>50</td>
<td>1.667</td>
</tr>
</tbody>
</table>

**Table 2:** Accuracy reading

<table>
<thead>
<tr>
<th>Level</th>
<th>Standard added (mg)</th>
<th>Standard recovered</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>40.5</td>
<td>40.43</td>
<td>99.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>50.8</td>
<td>50.28</td>
<td>99.97</td>
<td>99.37</td>
<td>0.4336</td>
</tr>
<tr>
<td>120%</td>
<td>60.1</td>
<td>59.68</td>
<td>99.30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3:** Deviation from recovery

<table>
<thead>
<tr>
<th>Level (Approx.)</th>
<th>Actual concentration (mcg/ml)</th>
<th>calculated (mg/ml) concentration</th>
<th>Accuracy (%)</th>
<th>% Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>40.5</td>
<td>40.43</td>
<td>99.83</td>
<td>-0.17</td>
</tr>
<tr>
<td>100%</td>
<td>50.8</td>
<td>50.28</td>
<td>99.97</td>
<td>-1.03</td>
</tr>
<tr>
<td>120%</td>
<td>60.1</td>
<td>59.68</td>
<td>99.30</td>
<td>-0.70</td>
</tr>
</tbody>
</table>

**Table 4:** Mean % Assay and %RSD

<table>
<thead>
<tr>
<th>CHEMIST A</th>
<th>CHEMIST B</th>
<th>AVERAGE</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>100.268</td>
<td>99.43</td>
<td>99.849</td>
<td>0.5935</td>
</tr>
</tbody>
</table>

**Table 5:** Validation Summary

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Acceptance criteria</th>
<th>Observation</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>PRECISION</td>
<td></td>
<td></td>
<td>COMPLIES</td>
</tr>
<tr>
<td></td>
<td>METHOD PRECISION</td>
<td>% RSD</td>
<td>0.9728</td>
<td>COMPLIES</td>
</tr>
<tr>
<td>02</td>
<td>SPECIFICITY</td>
<td>No considerable absorbance of any other component of formulation at λmax of analyte or at detection wavelength.</td>
<td>No absorbance observed at 314 nm</td>
<td>COMPLIES</td>
</tr>
<tr>
<td>03</td>
<td>ACCURACY (BY RECOVERY)</td>
<td></td>
<td></td>
<td>COMPLIES</td>
</tr>
<tr>
<td></td>
<td>% RECOVERY</td>
<td>100 ± 2 %</td>
<td>99.37 %</td>
<td>COMPLIES</td>
</tr>
<tr>
<td></td>
<td>% RSD</td>
<td>NMT 1.5 %</td>
<td>0.4366 %</td>
<td>COMPLIES</td>
</tr>
<tr>
<td></td>
<td>% DEVIATION FROM ACCURACY</td>
<td>± 1.5 %</td>
<td>80 %: -0.17</td>
<td>COMPLIES</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100 %: -1.03</td>
<td>COMPLIES</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>120 %: -0.70</td>
<td>COMPLIES</td>
</tr>
<tr>
<td>04</td>
<td>LINEARITY</td>
<td>COEFFICIENT OF CORRELATION (r²) NLT 0.995</td>
<td>r²: 0.995</td>
<td>COMPLIES</td>
</tr>
<tr>
<td>05</td>
<td>RUGGEDNESS</td>
<td>% RSD: NMT 1.5 %</td>
<td>% RSD: 0.5935</td>
<td>COMPLIES</td>
</tr>
<tr>
<td>06</td>
<td>LOD</td>
<td>-</td>
<td>5.24 mg</td>
<td>COMPLIES</td>
</tr>
<tr>
<td></td>
<td>LOQ</td>
<td></td>
<td>30.249 mg</td>
<td></td>
</tr>
</tbody>
</table>
Prepared according to the assay procedure. The results are shown in Table 2 & 3.

The percentage recovery for 6N was found in the range of 98.37 % with an overall %RSD of 0.4366. From the data obtained which was given in table 2, the method was found to be accurate.

### 3.3 Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogenous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard of a series of measurements. Assay Preparation & Standard Preparation were prepared as per method of analysis of 6N six assay sample preparations as per the experimental conditions in method of analysis. And calculated percent of 6N in each assay sample percent by spectrophotometry and the results and observation for system precision data as follows:

- 0.565 is average absorbance and % RSD 0.0968 and method precision assay 100.27 and % RSD was 0.972 .

### 3.4 Specificity

Specificity study is designed to prove that the 6N in the solution gives maximum absorbance at wave length 314 nm and there is no interference from solvent. The purpose of this study to establish the fact that inherent chemical stability of the molecule remains intact during its existence. If any degradation product formed, it can be monitored and resolved to quantify the nature and extent of degradation.

For this the spectrum of 6N, placebos are studied. The sample preparation is as per Methodology. Spectrum of 6N & placebo are shown in Fig 3.

For the spectrophotometric method no any other component of formulation shows considerable absorbance at the λ-max of the analyte or at the detection wavelength of subject analyte. In this case of 6N the detection wavelength is 314 nm.
nm. The placebo solution under same condition as that of assay conditions of 6N does not shows any absorbance at 314 nm.

3.5 Ruggedness

The ruggedness of analytical method is the degree of reproducibility of test results obtained the analysis of the sample on different days, by different chemist using different instruments.

In this study two individual assay sample preparation of 6N drug product were prepared by different Chemist for analysis. Six (6) replicate observations of the same standard solution were obtained as well as six observations of different sample solution were recorded. The assay percentage of each sample was calculated in each case. The results for chemist B system precision average absorbance are 0.564 and % RSD 0.6412 and method precision % assay is 99.43 and % RSD 0.3995 summarized in Table 4.

3.7 Limit of Detection and Limit of Quantitation

Limit of Detection (LOD): The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, not quantified.

Limit of Quantitation (LOQ): The lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method.

LOD:  3.3 (SD)/ Slope
       3.3 (0.5542)/ 0.349 [SD = 0.5542]
       5.24 mg

2. LOQ:  10 (SD)/ Slope
       10 (0.55226)/ 0.349
       = 30.249 mg

RESULTS AND DISCUSSION

The method was validated with respect to Linearity, Accuracy, Precision, Specificity, Robustness, Ruggedness, Limit of Detection (LOD) Limit of Quantitation (LOQ). The method was established according to ICH guidelines. 6N exhibited maximum absorption at 314 nm and obeyed Beer’s law in the concentration range of 10-50 µg/ml. The proposed method for the determination of 6N showed linear regression

\[ y = 0.349x + 0.107 \] with a coefficient correlation (r²) of 0.995 (Fig 1). The precision was determined by the relative standard deviation of the six-assay sample of 6N and assay of each was calculated the obtained relative standard deviation of assay % was less than 1.5%. The percentage recovery for 6N was found in the range of 98.97 % which indicates that developed method was simple, rapid and precise. LOD was found to be 5.24 mg and limit of Quantitation to be 30.249 mg. The proposed method will be suitable for the analysis of newly synthesized pyrimidine derivative (6N) in bulk dosage form.

REFERENCES: