Method development and validation of potent pyrimidine derivative by UV-VIS spectrophotometer

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Abstract

Background: A rapid and sensitive ultraviolet-visible (UV-VIS) spectroscopic method was developed for the estimation of pyrimidine derivative 6-Bromo-3-(6-(2,6-dichlorophenyl)-2-(morpholinomethylamino) pyrimidine-4-yl) -2H-chromen-2-one (BT10M) in bulk form.

Results: Pyrimidine derivative was monitored at 275 nm with UV detection, and there is no interference of diluents at 275 nm. The method was found to be linear in the range of 50 to 150 μg/ml. The accuracy and precision were determined and validated statistically. The method was validated as a guideline.

Conclusions: The results showed that the proposed method is suitable for the accurate, precise, and rapid determination of pyrimidine derivative.

Keywords: Pyrimidine; Derivative; UV-VIS spectroscopy; Validation

Background

Nitrogen containing heterocyclic ring such as pyrimidine is a promising structural moiety for drug design. Pyrimidine derivatives form a component in various useful drugs and are associated with many biological and therapeutic activities. Condensed pyrimidines have been reported as antimicrobial [1-3], anti-inflammatory [4,5], analgesic [6,7], anticancer [8-10], anti-HIV [11], antitubercular, antimalarial, diuretic, and cardiovascular disease [12] (Scheme 1).

The present work is a synthesis, a biological evaluation and validation of novel pyrimidine derivatives. Research workers have synthesized 50 pyrimidine derivatives (T1M-T10M, T1P-T10P, BT1M-BT10M, BT1P-BT10P, CT1M-CT5M, and CT1P-CT5P). Among them, BT10M exhibited maximum antimicrobial, anti-inflammatory, and analgesic activity. Hence, a validation study was done on BT10M. BT10M is chemically [6-Bromo-3-(6-(2,6-dichlorophenyl)-2-(morpholinomethylamino) pyrimidine-4-yl) -2H-chromen-2-one]. It is a yellow crystalline powder with a molecular formula of C24H19BrCl2N4O3 and a molecular weight of 562.24. It is a potent antimicrobial, analgesic and anti-inflammatory agent among all the synthesized derivatives. Hence, the aim of present investigation is to develop a simpler, rapid, and cost-effective analytical method for the determination of pyrimidine derivative (BT10M) in bulk dosage form suitable for routine quality control analysis.

Method validation is the process used to confirm that analytical procedure employed for a 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 [13].

Methods

Chemical and reagent

BT10M was synthesized by research workers and then validated. Methanol and acetonitrile (1:1) were used throughout spectrophotometric method development and validation.
Instrumentation
The method was performed on a double-beam ultraviolet-visible (UV-VIS) spectrophotometer (Shimadzu model 1700 (Shimadzu, Kyoto, Japan)) having two matched quartz cells with a 1-cm light path.

Determination of maximum wavelength ($\lambda_{\text{max}}$), methodology, and sample preparation
About 50 mg of BT$_{10}$M was weighed accurately and transferred into a 50-ml volumetric flask and dissolved in 25 ml of methanol and acetonitrile (1:1) and made up to the volume with the same solvent mixture to give a standard concentration of 1,000 $\mu$g/ml. Transfer 5 ml of above solution into the 50-ml volumetric flask, dilute, and made up to the volume with the same solvent mixture to get a standard concentration of 100 $\mu$g/ml. This solution was scanned against a blank over the entire UV-VIS wavelength of 200 to 400. Based on the spectrum, a $\lambda_{\text{max}}$ of 275 nm was selected for further analysis.

Table 1 Validation summary

| Serial number | Parameters                        | Acceptance criteria          | Observation                  |
|---------------|-----------------------------------|------------------------------|------------------------------|
| 01            | Precision                         |                              |                              |
|               | (a) System precision %RSD         | NMT 1.5%                     | 0.0968                       |
|               | (b) Method precision %RSD         | NMT 1.5%                     | 0.27995                      |
| 02            | Specificity                       | No considerable absorbance of any other component of formulation at $\lambda_{\text{max}}$ of analyte or at detection wavelength | No absorbance observed at 275 nm |
| 03            | Accuracy (by recovery)            |                              |                              |
|               | % Recovery                        | 100% ± 2%                    | 100.12%                     |
|               | %RSD                             | NMT 1.5%                     | 1.1777%                     |
|               | % Deviation from accuracy         | ±1.5%                        | 080%: +01.46                |
|               |                                  |                              | 100%: −00.77                |
|               |                                  |                              | 120%: −00.32                |
| 04            | Linearity                         | Coefficient of correlation ($r^2$) NLT 0.998 | $r^2$: 0.997            |
| 05            | Ruggedness                        | %RSD: NMT 1.5%               | %RSD: 0.1572                 |
| 06            | Robustness                        | %RSD: NMT 1.5%               | Original condition: +0.72 Changed condition: −0.82 |
| 07            | Limit of detection and limit of quantitation | LOD 145.2 mg | 145.2 mg                     |
|               |                                   | LOQ 440.00 mg                | 440.00 mg                    |

NMT, not more than; NLT, not less than.
Results
The method was validated with respect to linearity, accuracy, precision, specificity, robustness, ruggedness, LOD, and LOQ in Table 1.

Discussion
The method was validated with respect to linearity, accuracy, precision, specificity, robustness, ruggedness, LOD, and LOQ. The method was established according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines. BT\textsubscript{10}M exhibited maximum absorption at 275 nm and obeyed Beer’s law in the concentration range of 50 to 150 μg/ml. The proposed method for the determination of BT\textsubscript{10}M showed linear regression \( y = 0.005x + 0.025 \) with a coefficient correlation \( (r^2) \) of 0.997 (Figure 1). The precision was determined by the relative standard deviation of the six-assay sample of BT\textsubscript{10}M, and the assay of each was calculated and the obtained relative standard deviation of % assay was less than 1.5%. The percentage recovery for BT\textsubscript{10}M was found in the range of 98.97% to 99.83% which indicates that the developed method was simple, rapid, and precise. LOD was found to be 145.2 and limit of quantitation to be 440.0. The proposed method will be suitable for the analysis of newly synthesized pyrimidine derivative (BT\textsubscript{10}M) in bulk dosage form.

Experimental
Validation
The methods were validated with respect to linearity, accuracy, precision, specificity, ruggedness, robustness, limit of detection (LOD), and limit of quantitation (LOQ).

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 BT\textsubscript{10}M is 100 μg/ml. So the working range of analyte was set between 50, 62.5, 75, 87.5, 100, 112.5, 125, 137.5 and 150 μg/ml to show the linearity of the curve obtained. The observations and calibration curve are shown in Table 2 and Figure 1.

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 BT\textsubscript{10}M to the placebo at levels 80%, 100%, and 120% of working standard. The samples were prepared according to the assay procedure. The results are shown in Tables 3 and 4.

| Serial number | Approximate concentration (μg/ml) | Average absorbance at 275 nm |
|---------------|----------------------------------|----------------------------|
| 1             | 50.0                             | 0.299                      |
| 2             | 62.5                             | 0.361                      |
| 3             | 75.0                             | 0.458                      |
| 4             | 87.5                             | 0.511                      |
| 5             | 100.0                            | 0.569                      |
| 6             | 112.5                            | 0.651                      |
| 7             | 125.0                            | 0.714                      |
| 8             | 137.5                            | 0.803                      |
| 9             | 150.0                            | 0.866                      |
The percentage recovery for BT$_{10M}$ was found in the range of 98.97% to 99.83% with an overall relative standard deviation (%RSD) of 0.4366. From the data obtained which was given in Table 3, the method was found to be accurate. Formula of standard deviation was SD = (xi − x/n − 1)/2 if n is very large. In case of very small data, SD = (xi − x/n)1/2.

**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 and standard preparation were prepared as per method of analysis of six BT$_{10M}$ assay sample preparations as per the experimental conditions in method of analysis. Calculated percent of BT$_{10M}$ in each assay sample percent by spectrophotometry and the results and observation are summarized in Tables 5 and 6.

**Assay**

\[
\%\text{Assay} = \frac{\text{Abs. of Smp.}}{\text{Abs. of standard}} \times \frac{\text{Wt. of standard}}{\text{Dil. factor}} \times \frac{\text{Wt. of Smp}}{100}
\]

The precision will be determined by the relative standard deviation of the six-assay sample of BT$_{10M}$, and the assay of each is calculated and the obtained relative standard deviation of % assay should be less than 1.5%. The %RSD shows that precision of the method was satisfactory.

**Specificity**

Specificity study is designed to prove that the BT$_{10M}$ in the solution gives maximum absorbance at wavelength 275 nm, and there is no interference from the solvent. The purpose of this study is 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 BT$_{10M}$, placebos are studied. The sample preparation is as per methodology. The spectrum of BT$_{10M}$ is shown in Figure 2.

For the spectrophotometric method, no other component of formulation shows considerable absorbance at the $\lambda_{max}$ of the analyte or at the detection wavelength of the subject analyte. In this case of BT$_{10M}$, the detection wavelength is 275 nm. The placebo solution under the same condition does not show any absorbance at 275 nm.

### Table 3 Accuracy reading

| Level (Approximate) | Standard added (mg) | Absorbance at 275 nm | Standard recovered (mg) | %Recovery | Mean recovery | %RSD |
|---------------------|---------------------|----------------------|-------------------------|-----------|--------------|------|
| 80%                 | 40.5                | 0.458                | 40.43                   | 99.83     | 99.37%       | 0.4366 |
|                     |                     | 0.451                |                         |           |              |      |
|                     |                     | 0.459                |                         |           |              |      |
| 100%                | 50.8                | 0.567                | 50.28                   | 99.97     | 098.97       |      |
|                     |                     | 0.561                |                         |           |              |      |
|                     |                     | 0.574                |                         |           |              |      |
| 120%                | 60.1                | 0.670                | 59.68                   | 99.30     | 099.30       |      |
|                     |                     | 0.676                |                         |           |              |      |
|                     |                     | 0.673                |                         |           |              |      |

### Table 4 Deviation from recovery

| Level (Approximate) | Actual concentration (µg/ml) | Concentration calculated (mg/ml) | Accuracy (%) | % Deviation |
|---------------------|------------------------------|---------------------------------|--------------|------------|
| 80%                 | 080.75                       | 081.9289                        | 101.46       | +01.46     |
| 100%                | 100.40                       | 099.6269                        | 99.23        | −0.77      |
| 120%                | 121.05                       | 120.6626                        | 99.68        | −0.32      |

### Table 5 System precision data of BT$_{10M}$ working standard solution

| Serial number | Absorbance at 275 nm |
|---------------|----------------------|
| 1             | 0.566                |
| 2             | 0.565                |
| 3             | 0.565                |
| 4             | 0.566                |
| 5             | 0.566                |
| 6             | 0.565                |
| Average       | 0.565                |
| %RSD          | 0.0968               |

Weight of BT$_{10M}$ WS = 50.0 mg.
Ruggedness
The ruggedness of the analytical method is the degree of reproducibility of test results obtained by the analysis of the sample on different days, by different chemist using different instruments. In this study, two individual assay sample preparations of BT$_{10}$M drug product were prepared by different chemists 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 are summarized in Tables 5, 6, 7, 8, 9.

The ruggedness (inter-day precision) will be determined by the relative standard deviation of the results of assay of two different chemists on different days.

Robustness
The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability under normal usage. Method robustness was determined by analyzing the same sample at normal operating conditions and also by changing some operating analytical conditions. The result and observation are summarized in Table 10.

The robustness will be determined by the relative standard deviation of the results of assay of two different conditions by a change in original parameter.

Limit of detection and limit of quantitation
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 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.

\[
\text{LOD} : \frac{3.3 \times (\text{SD})}{\text{Slope}} = \frac{3.3 \times (0.200)}{0.005} \left[ \text{SD} = 0.2200 \right] = 145.2 \text{mg}
\]

\[
\text{LOQ} : \frac{10 \times (\text{SD})}{\text{Slope}} = \frac{10 \times (0.2200)}{0.005} = 440.00 \text{mg}
\]

Conclusions
All the validation parameters for all the developed methods were studied as per ICH guidelines. All the
methods were found to be accurate, simple, specific, selective, precise, and reproducible. Hence, the method can be used for routine analysis of BT$_{10}$M in bulk dosage form.

Competing interests
The authors declare that they have no competing interests.

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Table 9 Mean % assay and %RSD

| Chemist A | Chemist B | Average | %RSD |
|-----------|-----------|---------|------|
| 101.2683  | 101.0433  | 101.1558| 0.1572|

Table 10 Percentage deviation for sample under both conditions

| Parameter            | Original condition | Changed conditions |
|----------------------|---------------------|--------------------|
| Dilution medium      | Acetonitrile:methanol | Acetonitrile:methanol |
| Assay in %           | 101.98              | 100.44             |
| % Deviation from mean| +0.72               | –0.82              |

Table 9 Mean % assay and %RSD

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