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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-(morpolinomethylamino) pyrimidine4-yl) -2H-chromen-2-one ($BT_{10}M$) 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 (T_1M-$T_{10}M$, T_1P-$T_{10}P$, BT_1M-$BT_{10}M$, BT_1P-$BT_{10}P$, CT_1M-CT_5M, and CT_1P-CT_5P). Among them, $BT_{10}M$ exhibited maximum antimicrobial, anti-inflammatory, and analgesic activity. Hence, a validation study was done on $BT_{10}M$. $BT_{10}M$ is chemically [6-Bromo-3-(6-(2,6-dichlorophenyl)-2-(morpolinomethylamino) pyrimidine4-yl) -2H-chromen-2-one]. It is a yellow crystalline powder with a molecular formula of $C_{24}H_{19}BrCl_2N_4O_3$ 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 ($BT_{10}M$) 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

$BT_{10}M$ was synthesized by research workers and then validated. Methanol and acetonitrile (1:1) were used throughout spectrophotometric method development and validation.

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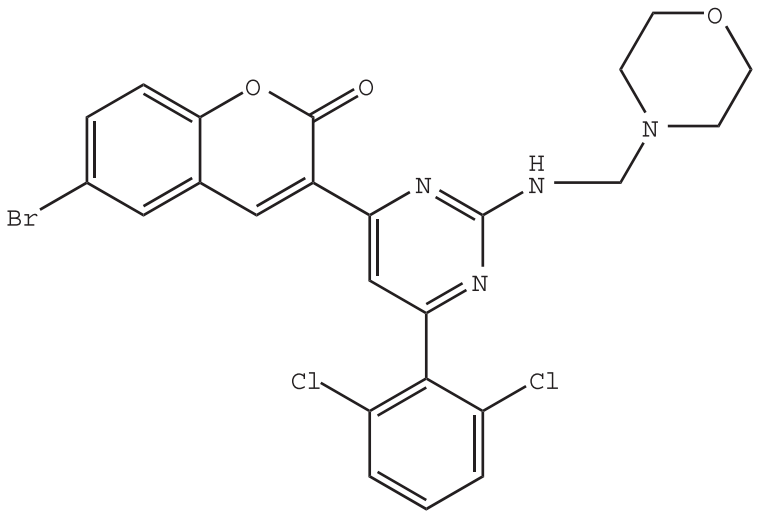

Scheme 1 Chemical structure of $BT_{10}M$.

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 (λ_{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 μg/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 μg/ml. This solution was scanned against a blank over the entire UV-VIS wavelength of 200 to 400. Based on the spectrum, a λ_{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 λ_{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: 0.997
		(r^2) NLT 0.998	
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
		LOQ	440.00 mg

NMT, not more than; NLT, not less than.

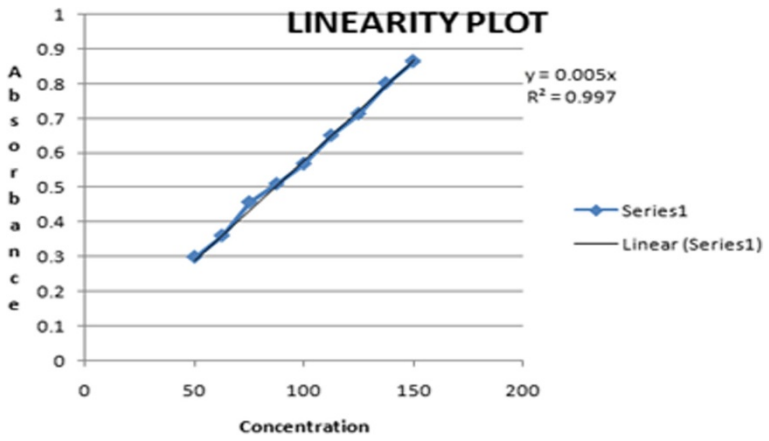

Figure 1 Linearity plot. The BT10M absorbance response in the concentration range of about 50 mcg/ml to 150 mcg/ml was found to be linear to the analyte concentration in the solution with a correlation coefficient (r2) of 0.997.

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. $BT_{10}M$ 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 $BT_{10}M$ 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 $BT_{10}M$, and the assay of each was calculated and the obtained relative standard deviation of % assay was less than 1.5%. The percentage recovery for $BT_{10}M$ 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 ($BT_{10}M$) 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 $BT_{10}M$ 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 $BT_{10}M$ 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.

Table 2 Linearity table of $BT_{10}M$ in working standard

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

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	098.97		
		0.561				
		0.574				
120%	60.1	0.670	59.68	099.30		
		0.676				
		0.673				

The percentage recovery for $BT_{10}M$ 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)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 $BT_{10}M$ assay sample preparations as per the experimental conditions in method of analysis. Calculated percent of $BT_{10}M$ in each assay sample percent by spectrophotometry and the results and observation are summarized in Tables 5 and 6.

Assay

$$\%\,\mathbf{Assay} = \frac{\text{Abs. of Smp.}}{\text{Abs. of standard}} \times \frac{\text{Wt. of standard}}{\text{Dil. factor}} \times \frac{\text{Dil. factor}}{\text{Wt. of Smp}} \times 100$$

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	099.23	−00.77
120%	121.05	120.6626	099.68	−00.32

The precision will be determined by the relative standard deviation of the six-assay sample of $BT_{10}M$, 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 $BT_{10}M$ 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 $BT_{10}M$, placebos are studied. The sample preparation is as per methodology. The spectrum of $BT_{10}M$ is shown in Figure 2.

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

Table 5 System precision data of $BT_{10}M$ 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 $BT_{10}M$ WS = 50.0 mg.

Table 6 Method precision data for estimation of $BT_{10}M$

Assay	Sample weight (mg)	Absorbance	% Assay
1	50.2	0.576	101.75
2	50.4	0.576	101.29
3	50.2	0.572	101.02
4	50.8	0.580	101.22
5	50.0	0.569	100.96
6	50.7	0.579	101.37
	Average		101.2683
	%RSD		0.27995

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.

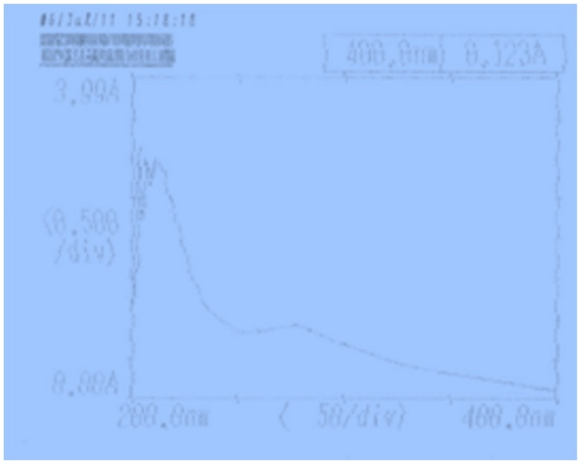

Figure 2 Spectrum of $BT_{10}M$.

Table 7 System precision data of $BT_{10}M$ working standard solution

Serial number	Absorbance at 275 nm
1	0.559
2	0.561
3	0.560
4	0.561
5	0.560
6	0.561
Average	0.560
%RSD	0.14571

Weight of $BT_{10}M$: 49.8 mg.

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.

$$\mathbf{LOD:}\ \mathbf{3.3(SD)/Slope}$$
$$\mathbf{3.3(0.200)/0.005}\ [\mathbf{SD = 0.2200}]$$
$$\mathbf{= 145.2\,mg}$$

$$\mathbf{2.LOQ:}\ \mathbf{10(SD)/Slope}$$
$$\mathbf{10(0.2200)/0.005}$$
$$\mathbf{= 440.00\,mg}$$

Conclusions

All the validation parameters for all the developed methods were studied as per ICH guidelines. All the

Table 8 Method precision data for $BT_{10}M$

Assay	Sample weight (mg)	Absorbance	% Assay
1	50.2	0.566	100.27
2	50.5	0.572	100.73
3	50.8	0.582	101.94
4	50.7	0.578	101.45
5	50.2	0.568	100.64
6	50.9	0.579	101.23
	Average		101.0433
	%RSD		0.6041

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	Acetonirile:methanol
	1:1	1:2
Assay in %	101.98	100.44
% Deviation from mean assay value obtained in precision studies	+00.72	−00.82

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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References
1. Mohmed MS, Awad SM, Sayed IA (2010) Synthesis of certain pyrimidine derivatives as antimicrobial agents and anti-inflammatory agents. Molecules 15:1882–1890
2. Munawar AM, Azad M, Siddiqui L (2008) Synthesis & antimicrobial study of some quinolinyl pyrimidine derivatives. J Chin Chem Soc 55:394–400
3. Ashraf HF, Abd ElW (2012) Synthesis, reactions and evaluation of the antimicrobial activity of some 4-(p-Halophenyl)-4H-naphthopyran, pyranopyrimidine and pyranotriazolopyrimidine derivatives. Pharmaceuticals 5:745–757
4. Nofal MZ, Emans Z, Wafaa EE (2011) Synthesis of pyrimidines and fused pyrimidines and their evaluation of antimicrobial activity. Acta Polnniae Pharmaceutica-Drug Res 68(4):507–517
5. Kota RK, Kompelly KK, Surampudi R, Kulkarni R (2011) Synthesis and anti-inflammatory activity of novel pyrazolo [3,4-d] pyrimidines. J Chem Pharm Res 3(4):848–853
6. Gupta JK, Sharma PK, Dudhe R, Chaudhary A (2011) Synthesis and analgesic activity of novel pyrimidine derivatives of coumarin moiety. Acta Polnniae Pharmaceutica-Drug Res 68(5):785–793
7. Sondhi SM, Dinodia M, Shukla R (2009) Synthesis, anti-inflammatory and analgesic activity evaluation of some pyrimidine derivatives. Indian J Chem 49B:273–281
8. Sharma S, Sharma PK, Kumar N, Dudhe R (2011) A review on various heterocyclic moieties and their antitubercular activity. Biomed Pharmacother 65:244–251
9. Al-abdullah ES (2011) Synthesis and anticancer activity of some novel tetralin-6-yl-pyrazoline, 2-thioxopyrimidine, 2-oxopyridine, 2-thioxo-pyridine and 2-iminopyridine derivatives. Molecules 16:3410–3419
10. EL-Zahar MI, Adb El-Karim SS, Haiba ME, Khedr MA (2011) Synthesis, antitumor activity and molecular docking study of novel benzofuran-2-yl pyrazole pyrimidine derivatives. Acta Polnniae Pharmaceutica-Drug Res 68(3):357–373
11. Mohamed YA, Mohamed SF, Abdullah MM (2012) Cytotoxicity and anti-HIV evaluations of some new synthesized quinazoline and thioxopyrimidine derivatives using 4-(thiophen-2-yl)-3,4,5,6-tetrahydrobenzo[h]quinazoline-2 (1H)-thione as synthon. J Chem Sci 124(3):693–702
12. Chaudhary A, Sharma PK, Verma PK, Kumar N, Dudhe R (2012) Microwave assisted synthesis of novel pyrimidine derivatives and investigation of their analgesic and ulcerogenic activity. Med Chem Res 21(11):3629–3645
13. Chaudhary A, Verma PK, Dudhe R (2012) Significant anti-microbial activity of novel pyrimidine derivatives. Elixir Pharmacy 45:7956–7963

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