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Original Article

VISIBLE SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF IMATINIB IN BULK AND FORMULATION

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ABSTRACT

Objective: A new, simple, sensitive, precise and reproducible UV visible spectrophotometric method was developed for the determination of Imatinib in pharmaceutical formulations with alizarin.

Methods: The method is based on formation of yellow-colored complex. The UV spectrum of Imatinib in methanol showed λ max at 431 nm. Beer's law is valid in the concentration range of 10-70 µg/ml. This method was validated for linearity, accuracy, precision, ruggedness and robustness.

Results: The method has demonstrated excellent linearity over the range of 10-70 µg/ml with regression equation y =0.013x-0.017 and regression correlation coefficient r^2= 0.997. Moreover, the method was found to be highly sensitive with LOD (4.3µg/ml) and LOQ (13.07µg/ml).

Conclusion: Based on results the proposed method can be successfully applied for the assay of Imatinib in various pharmaceutical dosage forms.

Keywords: Imatinib, Spectrophotometry, Alizarin, Method Development, Validation

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INTRODUCTION

Cancer is an abnormal growth of cell which tends to proliferate in an uncontrolled way and in some cases metastasize. It is the common cause of mortality [1].

Imatinib is an anticancer agent used to treat leukemia. Specifically, it is used for chronic myelogenous leukemia (CML) and acute lymphocytic leukemia (ALL), certain types of gastrointestinal stromal tumors (GIST), chronic eosinophilic leukemia. Specifically, Philadelphia chromosome-positive (Ph+) [2-5].

The activity of tyrosine kinase i.e. multiplication of cell is blocked by Imatinib. This will lead to a stoppage of the spreading of cancer cell [6-8]. The United states approved Imatinib as medical use in 2001. It is also included in the World Health Organization List of Essentials Medicines, the most effective and safe medicines needed in a health system. Imatinib was drug to be pushed for approval of designation by FDA [9-13].

Structure of imatinib

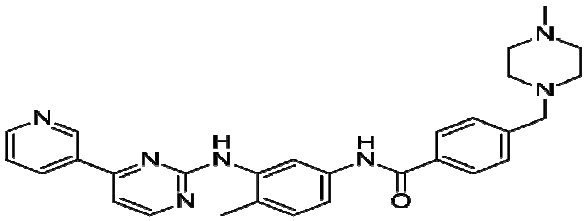

Fig. 1: The Chemical name of Imatinib is α–(4-methyl-1-piperazinyl)-3 [4–(3-pyridyl)–2-pyrimidinyl)-p-toluidide. The molecular formula of Imatinib is $C_{29}H_{31}N_7O$ and molecular weight is 493.603 gm/ml. Imatinib is white powder and has melting point 214 °-224 °C. It is freely soluble in distilled water and methanol [14-17]

The aim of this work is to introduce a simple, precise and rapid procedure for the simultaneous quantitation of the cited drug in plasma and pharmaceutical formulation.

MATERIALS AND METHODS

Materials

Imatinib was taken as a gift sample from Microlab, Bengaluru, India. Alizarin, Methanol and Dichloromethane were used were of analytical grade.

Instruments

A UV visible single beam spectrometer [systronics 119] and Shimadzu 1800-UV spectrophotometer with 1 cm quartz cuvettes were used for all absorbance measurement.

All weights were taken on an analytical balance (Shimadzu AY220). Sonicator was used for dissolving Imatinib in methanol.

Experimental

Preparation of alizarin

Alizarin 0.1 % (w/v) was dissolved in the least amount of methanol and completed to the required volume using dichloromethane.

Preparation of standard stock solution

Accurately weighed 10 mg of Imatinib transferred to 100 ml volumetric flask. It was dissolved in methanol and sonicated for 10 min. The volume was made up to mark with methanol to obtain final strength.

Procedure for plotting a calibration curve

Into a series of 10 ml volumetric flasks, 1-7 ml of standard solution was pipetted out separately and to each flask, 1 ml of 0.1 % alizarin was added. The volume was completed to the mark using methanol. The developed yellow color was measured at wavelength 431 nm against blank solution prepared in a similar manner excluding a drug.

Analysis of imatinib in pharmaceutical dosage form

20 Capsules containing Imatinib were weighed. An accurately weighed portion of the powder equivalent to 10 mg of Imatinib was dissolve in a 100 ml of methanol and mixed for about for 5 min and sonicated for about 10 min then filtered. From formed solution with a concentration of 100µg/ml seven aliquots were pipetted out into a

100 ml of volumetric flask having concentration 10-70µ/ml to each flask and 0.5 ml of 0.1% alizarin was added. The volume was made up to mark with methanol. These solutions were analyzed at selected wavelength 431 nm and results were statistically validated.

RESULTS AND DISCUSSION

The absorption spectral analysis shows the λ max of Imatinib to be 431 nm.

UV Visible spectra of imatinib

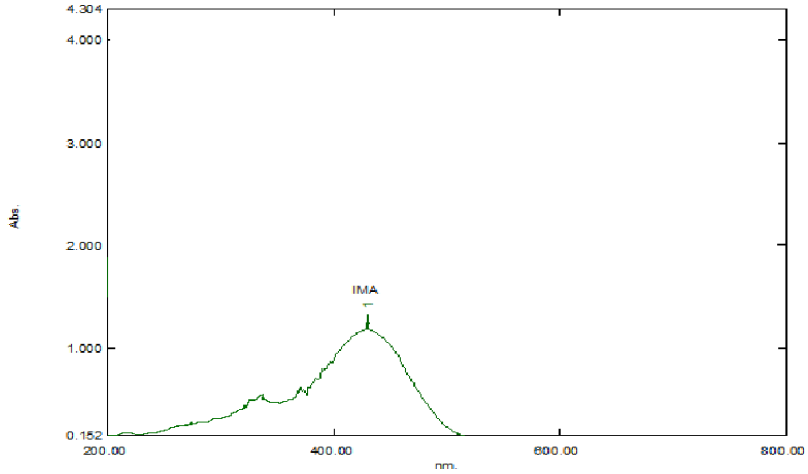

Fig. 2: The proposed method was validated according to ICH Q28 R1 guidelines for validation of analytical procedure [18-21]

Linearity

Calibration curve was analyzed on a single day. The level of quality control was assayed once with standard curve. The linear regression was used to plot the peak of absorbance of Imatinib vs concentration of Imatinib. The evaluation of variance with respect to concentration, slope, intercept and correlation coefficient were calculated for standard curve. Graph of linearity studies were plotted.

Table 1: Results for linearity

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.129
2	20	0.253
3	30	0.397
4	40	0.510
5	50	0.649
6	60	0.784
7	70	0960

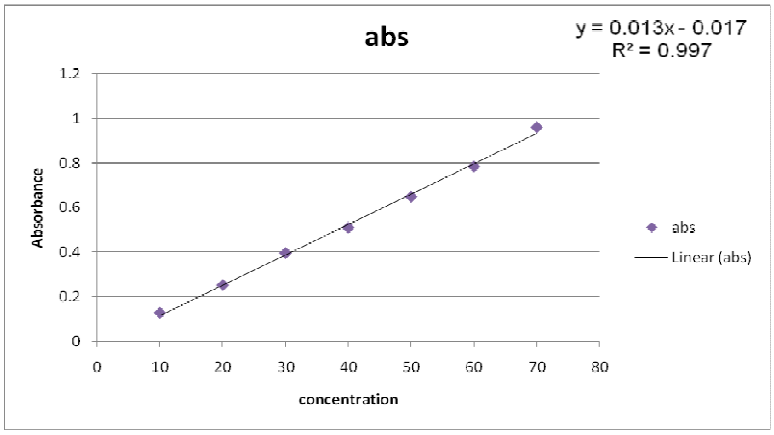

Fig. 2: Calibration curve for imatinib

Accuracy

The accuracy of an analytic method is the closeness of the test result obtained by that method to the true value. To perform the accuracy of proposed method standard addition method is used. Previously analyzed samples of Imatinib were added with standard drug solution and are analyzed by the proposed method. Recovery (%) RSD (%) was calculated for each concentration.

Range

The parameter of analytical method validation range is an interval between the lower and upper concentration limit of the analyte. The

range of this procedure is 10-70 µg/ml and it is selected on the trial and error basis.

Precision

Intra and inter-day precision were evaluated at 10-70 µg/ml. Seven replicates of each concentration were assayed in one run for the intra-day experiment. Six replicates of each concentration were assayed within 3 different days for inter-day experiments. The mean Imatinib value was found to be 30µg/ml. The RSD of intra-day precision was found to be 0.62 % and of inter-day was found to be 0.58 % suggesting that the developed method isprecise.

Limit of detection (LOD)

The Limit of Detection (LOD) of an analytical method is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated, under the stated experimental condition. It is calculated by formula,

$$LOD = 3\ Sa/b$$

Limit of quantitation (LOQ)

The parameter limit of quantitation is the lowest amount of drug in a solution which can be estimated with acceptable accuracy and precision under the experimental conditions.

$$LOQ = 10\ Sa/b$$

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same sample under a variety of condition such as different laboratories, different analyst, etc. Ruggedness is normally expressed as a lack of influence on the test results of operational and environmental variables of the analytical method.

Robustness

After deliberate variations in stated experimental conditions, the given analytical method is subjected for the reliability of an analysis. Typical variations are temperature, the stability of analytical solution etc.

Table 2: Regression analysis of the calibration curve for the proposed method

Parameters	Method values
λ max	431 nm
Beer's law	10-70 µg/ml
Correlation coefficient (r)	0.997
Regression equation (Y = mx+c)	0.013x-0.017
Slope (m)	0.013
Intercept (c)	-0.017
LOD(µg/ml)	4.3
LOQ(µg/ml)	13.07

Table 3: Result for precision (Intra-day)

S. No.	Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	% RSD
1	30	0.397	0.404	0.400	
2	30	0.395	0.400	0.396	
3	30	0.397	0.399	0.394	
4	30	0.400	0.402	0.399	
5	30	0.396	0.398	0.395	
6	30	0.395	0.396	0.394	
%RSD		0.46 %	0.71%	0.71 %	0.62 %

Table 4: Result for precision (Inter day)

S. No.	Concentration (µg/ml)	Day 1	Day 2	Day 3	%RSD
1	30	0.397	0.394	0.398	
2	30	0.395	0.399	0.400	
3	30	0.397	0.402	0.401	
4	30	0.400	0.394	0.398	
5	30	0.396	0.397	0.395	
6	30	0.395	0.398	0.397	
%RSD		0.46 %	0.77 %	0.53%	0.58%

Table 5: Result for robustness

Temperature	30°C	25°C
Concentration	7 µg/ml	7µg/ml
Absorbance	0.397	0.410
	0.395	0.406
	0.400	0.399
	0.397	0.395
	0.396	0.399
	0.395	0.397
Average	0.396667	0.401
SD	0.001862	0.005762

Table 6: Result of ruggedness

Concentration	Analyst 1	Analyst 2
7µg/ml	0.397	0.395
	0.395	0.397
	0.400	0.394
	0.397	0.398
	0.396	0.394
	0.395	0.400
Average	0.396667	0.396333
SD	0.001862	0.002422

Linearity

The wavelength was found at 431 nm. Seven concentrations were taken for linearity i.e. 10-70 µg/ml. Hence correlation coefficient (r^2) was found to be 0.997(table 1 and2). The absorbance was found within limit i.e. 0-2. Hence the performed parameter was found to be validated.

Precision

Intra-day precision

Intra-day precision study was carried out in 30 µg/ml concentration and the relative standard deviation was found be within limit i.e. less than 2%. Hence the performed parameter was validated (table 3).

Inter-day precision

The inter-day precision study was performed and the results showed to be less than 2% using 30 µg/ml concentration. Hence the performed parameter was validated (table 4).

Robustness

The results obtained by changing the concentration i.e. 7µg/ml and temperature i.e. 25°C and 30°C does not affect the results. The percentage relative standard deviation was found within the limit i.e. less than 2 %. Hence the parameter was found to be validated (table 5).

Ruggedness

The change in analyst at a concentration of 7µg/ml showed that the obtained result does not affected by it (table 6).

Limit of detection

The limit of detection was found to be 4.3µg/ml (table 2).

Limit of quantification

The limit of quantification was found to be 13.07µg/ml (table 2).

CONCLUSION

An analytical zero order derivative UV spectrophotometric method was developed and validated thoroughly for the quantitative determination of Imatinib in pure drug and capsule. The presented method was found to be rugged, simple, accurate, precise, reproducible and gives an acceptable recovery of the analyte, which can be directly easily applied to the analysis of the pharmaceutical capsule formulation of Imatinib.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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