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Research Article

DEVELOPMENT OF ANALYTICAL METHOD FOR IMATINIB MESYLATE BY ULTRAVIOLET SPECTROSCOPY

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ABSTRACT

Objective: A simple, selective, sensitive, specific, and spectrophotometric method has been developed for the detection of imatinib mesylate in pure form and formulations.

Methods: The analytical condition was optimized for the drug, carried out as per the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines.

Results: The drug shows absorption at 232.0 nm and obeyed beers law in the wide concentration range from 0.5 to 4.0 µg/ml. The lower limit of detection was found to be 0.331 µg/ml and the limit of quantification to be 1.004 µg/ml. The regression equation was found to be y = 0.08x. The precision of the method was found to be 99.04%±0.527% and the percentage of drug recovered by this method is 100.13%±1.375%.

Conclusion: The method is simple and suitable for determination for imatinib mesylate in pure and pharmaceutical preparation.

Keywords: Spectrophotometer, Imatinib mesylate, Determination.

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INTRODUCTION

Imatinib mesylate is a prescribed cancer drug for the treatment of leukemia and gastrointestinal tumors. It works by inhibiting cancer cell growth-related proteins to relieve symptoms, prevent cancer cells from spreading, and help other treatments. Imatinib mesylate is one of the newest anticancer drugs on the market and was one of the first drugs to be pushed through the Food and Drug Administration quick track approval designation. The drug is designed to be inhibitors of Bcr-Abl tyrosine kinase inhibitors and is used to treat chronic myeloid leukemia and gastrointestinal stromal tumors.

Imatinib mesylate's chemical name is 4-4[(4-methyl-1-piperazinyl) methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]phenyl]-benzamide mono methanesulfonate. It has $C_{29}H_{31}N_7O.CH_4O_3S$ molecular formula and a 589.71 molecular weight. It has the structural formula which is presented in Fig. 1. Imatinib mesylate is a white crystalline powder distilled water, 0.1 N HCl, methanol, and dimethyl ether sparingly soluble [1]. Review of literature reveals that only a few methods such as ultraviolet (UV) [2-7], colorimetery [8,9], biological fluid using high-performance liquid chromatography (HPLC) [10], reversed phase-HPLC [3,11-13], LC [14-16], LC-mass spectrometry [17] and ultrafast LC [18] were developed for the determination of imatinib mesylate in pure and pharmaceutical preparation.

MATERIALS

Instruments

Absorption spectral measurements were carried out with a Systronics 2202 UV-visible spectrophotometry and for sonication Branson 2510 Sonicator was used.

Chemicals

Imatinib mesylate obtained as a gift sample from Aspen Biopharma, Hyderabad, Telangana. Imatinib mesylate tablet formulations (100 mg) were procured from local pharmacies. Hydrochloric acid was of AR grade from Nice Pharmaceuticals Pvt. Ltd., and in house produced distilled water was used.

METHODS

Preparation of stock solution

About 100 mg of mg imatinib mesylate drug in pure form was weighed quantitatively and transferred to a 100 ml standard measuring flask, 0.1 M HCl was added to the above flask, dissolved, and sonicated for 15 min, and the capacity was made up to100 ml with 0.1 M HCl to obtain a final concentration of 1 mg/ml.

Determination of absorbance maxima

An appropriate aliquot portion of 2 ml of imatinib mesylate from a stock solution of imatinib mesylate was moved to 100 ml standard measuring flask, mixed with 0.1 N HCl and the volume was made up to 100 ml with 0.1 N HCl to obtain the concentration 20 µg/ml of imatinib mesylate. Drug solutions were scanned in spectrophotometry and the absorbance maxima were determined.

Validation of the proposed method

The anticipated method was authenticated as per the International Council for Harmonisation Q2 (R1) guidelines for linearity, range, accuracy, precision, stability, limit of detection (LOD), and limit of quantification (LOQ) [19].

Linearity and range

An appropriate aliquot portion of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 ml of imatinib mesylate from standard solution was transferred to 100 ml standard measuring flasks and dissolved in 0.1 M HCl volumes were made up to 100 ml with same solvents to obtain 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 µg/ml concentrations of imatinib mesylate. The absorbance of all the subsequent solutions was measured at 232.0 nm. The calibration curve was constructed by plotting drug concentration versus absorbance.

Precision

Ten tablets were weighed accurately and crushed into a fine powder. An accurately weighed quantity of powder equivalent to 100 mg of imatinib mesylate was transferred to 100 ml volumetric flask. About 25 ml of 0.1 M HCl was added and sonicated for 5 min, made up the volume with 0.1 M HCl and filtered. The resulting filtrate was measured at 232.0 nm using spectrophotometry.

Accuracy

A working standard solution of imatinib mesylate was prepared with 0.1 M HCl in a concentration of 2 mg/ml. Equivalent to 50 mg of imatinib mesylate (about 99 mg of tablet powder) weighed accurately and moved into three different 100 ml standard measuring flasks. About 25 ml of 0.1 N HCl was added and 12.5 ml (50%), 25 ml (100%), and 37.5 ml (150%) of the standard solution were added which contains 2 mg/ml of imatinib mesylate. The solution was sonicated for 5 min, made up the volume up to 100 ml with 0.1 N HCl. The resulting solutions were filtered separately and the filtrate of the solution was used to measure the absorbance at 232.0 nm using 0.1 N HCl as solvent blank. It was repeated for three times of different weighing at each level so that nine different weighings were performed.

LOD

The detection limit of a distinct analytical practice is the lowest amount of substance in a mixture which can be detected but not essentially quantitated as an exact value.

Limit of quantitation

The quantitation limit of a distinct analytical practice is the lowest analyte in a sample which can be quantitatively quantified with suitable precision and accuracy.

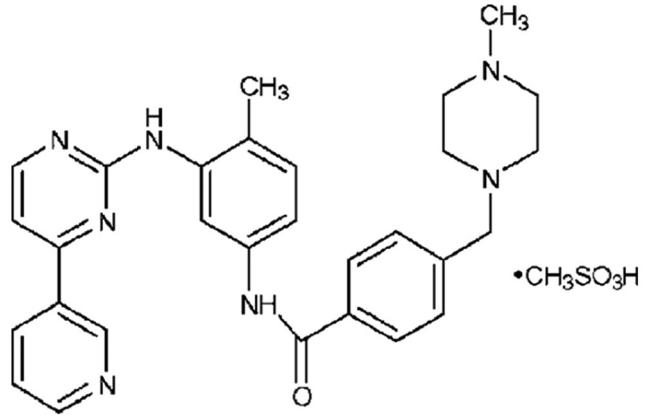

Fig. 1: Structure of imatinib mesylate

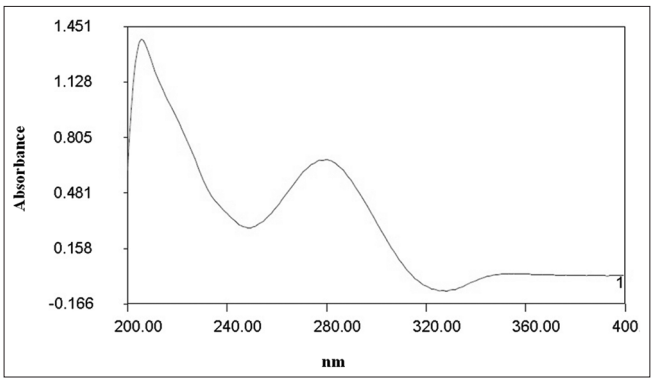

Fig. 2: Ultraviolet-spectrum of imatinib mesylate

Ruggedness

Ruggedness is a degree of reproducibility of test outcomes under normal, predictable operational situations from laboratory to laboratory and from analyst to analyst. Ruggedness is determined by the analysis of aliquots by a different analyst. About 2 µg/ml solutions were prepared and analyzed using a spectrophotometer.

Robustness

The robustness of an analytical practice is a measure of capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was carried out by changing the wavelength by spectrophotometry with 1 nm difference in wavelength at 231 nmm and 233 nm, respectively.

Stability study

Stability study is an integral part of much analytical procedure. The tests are based on the concept that the equipment, electronics, analytical operation, and samples to be analyzed constitute an integral system that can be evaluated as such. Stability test parameter is to be established for a particular procedure depends on the type of procedure being validated. It was carried out by 2 µg/ml solution which was prepared and measured for 2 h with the interval of 30 min at 232.0 nm using spectrophotometer.

RESULTS AND DISCUSSION

Determination of absorption maximum

Imatinib mesylate is a UV absorbing fragment with definite chromophores in the structure that absorbs at a specific wavelength and this fact was effectively used for their quantitative determinations using the UV spectroscopic method. An absorbance maximum was determined in spectrophotometry by taking 2 µg/ml imatinib mesylate drug which is dissolved in 0.1 N HCl and scanned from 200 to 400 nm using UV-visible spectrophotometer. The absorption spectra presented in Fig. 2. The spectral analysis showed that the λ max of imatinib mesylate was found to be at 232.0 nm in 0.1 N HCl.

Validation of the proposed method

Linearity and range

Calibration standards for imatinib mesylate covering a range of 0.5–4 µg/ml were prepared in serial dilutions that were made with

Table 1: Linearity of imatinib mesylate

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	0.50	0.046
2.	1.0	0.192
3.	1.5	0.127
4.	2.0	0.169
5.	2.5	0.201
6.	3.0	0.236
7.	3.5	0.271
8.	4.0	0.319

Table 2: Precision of imatinib mesylate

S. No.	Weight of the tablet powder (mg)	Absorbance	Drug content present (mg)	Percentage found
1.	0.1977	0.168	99.40	99.40
2.	0.1974	0.167	98.81	98.81
3.	0.1976	0.168	99.40	99.40
4.	0.1975	0.166	98.22	98.22
5.	0.1978	0.168	99.40	99.40
Mean				99.046
SD				0.527
RSD				0.0053

Table 3: Recovery of imatinib mesylate

S. No.	Percentage level	Sample weight (mg)	Drug in the tablet powder (mg)	Pure drug added (mg)	Total drug content (mg)	Absorbance	Amount found (mg)	Amount recovered	Percentage recovery
1.	50	0.1002	50	25	75	0.128	77.5	27.5	102.8
2.	50	0.1006	50	25	75	0.122	74.5	22.9	99.2
3.	50	0.1007	50	25	75	0.121	73.5	23.3	99.4
4.	100	0.1001	50	50	100	0.164	98.3	48.3	98.6
5.	100	0.1005	50	50	100	0.165	100	50	100.0
6.	100	0.1007	50	50	100	0.169	102	52	102.0
7.	150	0.1004	50	74	124	0.204	123.5	73.5	99.3
8.	150	0.1003	50	74	124	0.206	124	74	100.0
9.	150	0.1004	50	74	124	0.203	123	73	99.9
Mean									100.13
SD									1.375
RSD									0.0214

Table 4: Ruggedness of imatinib mesylate

S. No.	Absorbance at 232 nm	
	Analyst-1	Analyst-2
1.	0.179	0.177
2.	0.175	0.174
3.	0.170	0.171

Table 5: Robustness of imatinib mesylate

S. No.	Absorbance (231 nm)	Absorbance (233 nm)
1.	0.178	0.177
2.	0.175	0.176
3.	0.171	0.170

Table 6: Stability study of imatinib mesylate

S. No.	Time (h)	Absorbance (nm)
1.	0 min	0.179
2.	30 min	0.175
3.	1 h	0.170
4.	2 h	0.091

Table 7: Validation profile of imatinib mesylate

Parameters	Values
Linearity range (µg/ml)	0.5–4
Precision (%)	99.04±0.527
Accuracy (%)	100.13±1.375
50%	100.46±2.023
100%	100.2±1.70
150%	99.73±0.378
LOD (µg/ml)	0.331
LOQ (µg/ml)	1.004

LOD: Limit of detection, LOQ: Limit of quantification

0.1 N HCl. The absorbance of all resulting concentrations was measured at 232 nm. The graph between the concentration and absorbance was plotted. The regression equation was found to be y = 0.08x. The correlation coefficient (R^2) of the standard curve was found to be 0.993. The obtained data are presented in Table 1. It was found to be linear and hence suitable for the estimation of the drug.

Precision

The results of the precision data were presented in Table 2 for spectrophotometry. The values obtained in the repeatability (precision) shows that there is no significant difference in the precision value. Hence, the developed method can be used to analyze the imatinib mesylate in the tablet formulation. There is no evidence of interference of excipients with imatinib mesylate drug.

The mean precision value was found to be 99.046±0.53%. The value was obtained from 98.22% to 99.4% by spectrophotometric method.

Accuracy

From the data, drug – excipients interactions and/or drug – solvent interactions have not been observed. Since the standard deviation is less than 2% and the mean was above 100%, it was confirmed that there is no interference of any component as excipient is observed by this method. The three level accuracy data is presented in Table 3.

The percentage of recovery was found to be 100.13±1.37%. The value was obtained from 98.6% to 102.85% by spectrophotometric method.

LOD and LOQ

The LOD was found to be 0.331 µg/ml and the LOQ concentration was found to be 1.004 µg/ml.

Ruggedness

Ruggedness data are presented in Table 4 by the spectrophotometric method, which do not show any significant difference in the absorbance. Hence, the developed method is rugged.

Robustness

There is no significant difference in absorbance observed when the minor changes like the one nanometer difference in spectrophotometric estimation. The observed data were presented in Table 5.

Stability study

The data show (Table 6) that until the end of 1.5 h, there is no significant difference observed. At the same time after the time within 2 h, the absorbance was drastically reduced. Hence, it was inferred that the sample solution may be stable up to 1.5 h from the preparation of the sample solution.

Validation profile

Performing replicate analysis of the standard solutions was used to assess the accuracy and precision and reproducibility of the proposed methods. The selected concentration for the drug imatinib mesylate within the calibration range was prepared in 0.1 N HCl and analyzed with the relevant calibration curves to determine the intra-day and inter-day variability. The intra-day and inter-day precision were determined and presented in Table 7.

Estimation of imatinib mesylate by the developed method ensures the selective method than the other methods. The other methods require costly instrument which incurs the cost of analysis and the UV method which has also published do have other solvents. Stability of the solution

also found to be significantly acceptable range for the routine analysis. This method shows a wide range for linearity and most effective method to determine the said drug in the formulation and as pure form.

CONCLUSION

Spectrophotometric method for quantifying imatinib mesylate in pure and formulation has been developed and validated. The developed method is selective, precise, accurate, and linear over the concentration range from 0.5 to 4 µg/ml. The precision was found to be 99.04%±0.527%. The percentage of drug recovered 100.13%±1.375%. The LOD and LOQ were found to be 0.331 µg/ml and 1.004 µg/ml, respectively, with 0.1 M HCl. The developed method is simple and suitable for determination for imatinib mesylate in pure and pharmaceutical preparations.

AUTHORS' CONTRIBUTIONS

The authors have contributed to bringing the article by P. Ajithkumar and Dr. A. Anton Smith, where Ajithkumar performed the analysis and Dr. A. Anton Smith collected the data, designed the work, and drafted the article and critical revision of the article.

CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

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