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Reverse Phase High Performance Liquid Chromatography Determination of Imatinib Mesylate α-Form in Bulk and Pharmaceutical Formulations

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A rapid, precise, economical and accurate HPLC method for estimation of mephedrone in bulk and formulations was developed and validated. The chromatographic resolution of imatinib mesylate α-form was achieved using acetonitrile: 0.1M potassium dihydrogen phosphate buffer, (85:15 v/v) as a mobile phase UV detection at 254 nm and hypersil C_{18} column flow rate 1 mL/min. Recovery of Imatinib mesylate α-form from its formulation dosage form was greater than 99.48 %. Calibration curve was linear (r^2 = 0.9984) over the concentration ranging from 10 to 60 µg/mL of imatinib mesylate. The method has an accuracy of greater than 99 % and limit of detection and limit of quantification of 0.83300 µg/mL and 2.77400 µg/mL respectively. Results of the analysis were validated statistically and recovery studies were found to be satisfactory.

Key Words: Imatinib mesylate α-form, Chronic myelogenous leukemia, Reverse phase HPLC.

INTRODUCTION

Imatinib mesylate α-form (Fig. 1) is used in treating chronic myelogenous leukemia, gastrointestinal stromal tumors and some other diseases. In chronic myelogenous leukemia, the tyrosine kinase enzyme ABL is stuck in the on position; Imatinib binds to the site of tyrosine kinase activity and prevents its activity. One study demonstrated that imatinib mesylate was effective in patients with systemic mastocytosis, including those who had the D816V mutation in c-Kit experience has shown. However, that imatinib is much less effective in patients with this mutation and patients with the mutation comprise nearly 90 % of cases of mastocytosis. Early clinical trials also show its potential for treatment of hypereosinophilic syndrome and dermatofibrosarcoma protuberance. It is the first member of a new class of agents that act by specifically inhibiting a certain enzyme that is characteristic of a particular cancer cell, rather than non-specifically inhibiting and killing all rapidly dividing cells and served as a model for other targeted therapy modalities through tyrosine kinase inhibition.

A few publications are available for imatinib mesylate, some of are available on spectrophotmetric UV-Visible[1], LC MS and clinical validations[2-5]. Two HPLC methods[6] were also reported, one of it is in plasma and tissues and another one is on rat's urine. One expensive RP-HPLC is also reported for 1*H* & 2*H* forms. But none of them are employed an economical, precise and accurate RP-HPLC method for its α-form. The present authors are interested in developing a new method for determination of imatinib α-form in bulk and pharmaceutical dosage forms, which utilizes a economically cheap solvent system on Hypersil ODS C_{18} analytical columns. This kind of method effective to produce better retentions, very sharp and symmetrical peak shapes and exhibit very good sensitivity for imatinib mesylate α-form.

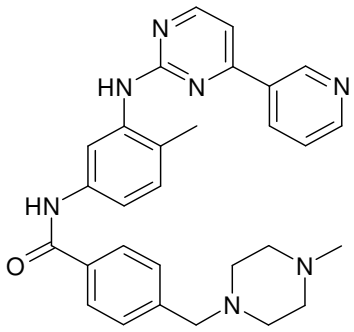

Fig. 1. Chemical structure of imatinib mesylate α-form

EXPERIMENTAL

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC20AT and LC20AT VP series HPLC

pumps, with a 20 µL injection of sample loop (manual) and SPD20 A VP UV-Visible detector. The out put signal was monitored and integrated using Shimadzu Class VP version 6.12 SP1 software. Hypersil, thermo, C_{18} (250 × 4.6 µ, 5 µ) column was used for separation.

Standards and reagents: Imatinib mesylate α-form and its formulation capsules were purchased from natco pharmacy and standard sample was gifted by Chandra labs. Acetonitrile, potassium dihydrogen phosphate were HPLC grade Merck chemicals, which are highly purified and their purities not less than 99.8 % purity.

Preparation of standard drug solutions: 50 mg each of imatinib mesylate α-form and weight of the formulation equivalent equivalent to 50 mg of the pure drug were accurately weighed and transferred into two separate 50 mL of volumetric flasks containing 25 mL of mobile phase and sonicated for 10 min, diluted with mobile phase up to the lower meniscus mark and filtered the solution through 0.45 µ membrane filter (1 mg/mL).

Chromatographic conditions: The mobile phase used in this study was a mixture of acetonitrile and phosphoric acid (buffer pH-4) 85:15 v/v, then the contents were solicited for 0.5 h for degassing purpose and then filtered through 0.45 µ (pore diameter) filter. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0 mL/min. the eluents were monitored at UV λ_{max} 254 nm. The column temperature was maintained ambient through out the experiment.

Calibration of standards: Calibration standards were prepared by spiking working standard into 25 mL volumetric flask to yield concentrations of 10, 20, 30, 40, 50 and 60 µg/mL. The final volume was made up to the mark by diluting with mobile phase. The represented data was shown in Table-1. In order to record the chromatogram 20 µL aliquot was injected into the analytical column and the resultant peak areas of the drug. Calibration curve was plotted between peak area ratios of drug against concentration of the drug.

TABLE-1
LINEARITY RANGE OF IMATINIB MESYLATE α-FORM

Conc. (µg/mL)	Peak area ratio	Statistical analysis
10	368.264	
20	627.035	Slope(1) : 310.3
30	924.810	
40	1271.854	Intercept: 26.664
60	1592.796	Correlation coefficient : 0.9984
80	1891.484	Asymmetric factor : 1.846

Recovery of imatinib mesylate α-form from its formulation: Finely powdered formulation dosage was accurately weighed equivalent to 50 mg imatinib mesylate α-form and was extracted with acetonitrile in to a 50 mL volumetric flask using ultra sonicator. This solution was diluted with mobile phase, so as to obtain a concentration in the range of linearity previously determined. All determinations were carried out in five replicates. The represented data was shown in Table-2.

TABLE-2
AMOUNT OF IMATINIB MESYLATE α-FORM IN FORMULATION TABLET BY HPLC METHOD

Formulation tablets (mg)	Labeled amount (mg)	Recovered amount (mg)	Recovery (%)
100 mg tablet	100	99.10	99.10

*Each value is the average of five determinations

RESULTS AND DISCUSSION

Specificity and selectivity of the method was assessed by preparing a drug concentration of 100 µg/mL from pure drug standard and commercial formulations in selected mobile phase were analyzed. The HPLC chromatograms (Figs. 2 and 3) were recorded for the drug matrix showed almost no other peaks within a retention time range of 7 min. Thus the HPLC method developed in this study is selective for imatinib mesylate α-form. The developed method is linear in the concentration range 10 to 60 µg/mL of the drug (Fig. 4). Intra-day and inter-day precision were studied by five replicate measurements at three different concentration levels over a period of 3 consecutive days. Accuracy of the method was determined by calculating recovery studies. Statistical evalution revealed that relative standard deviation (% RSD) of the drug at different concentration levels for five injections was less than 0.299. Precision and accuracy data were shown in Tables 3 and 4, respectively. For system suitability, five replicates of standard sample were injected and different parameters were studied (Table-5). The tailing factor for imatinib mesylate α-form was always less than 2.

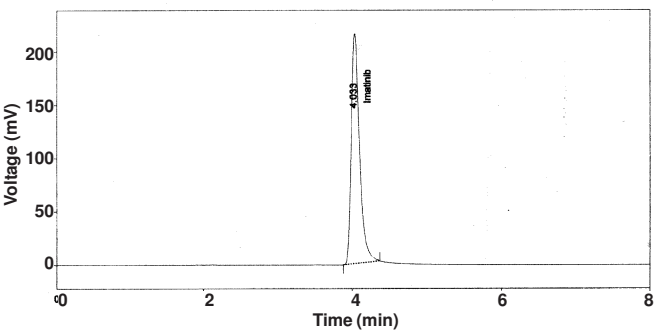

Fig. 2. HPLC chromatogram of imatinib mesylate α-form (standard)

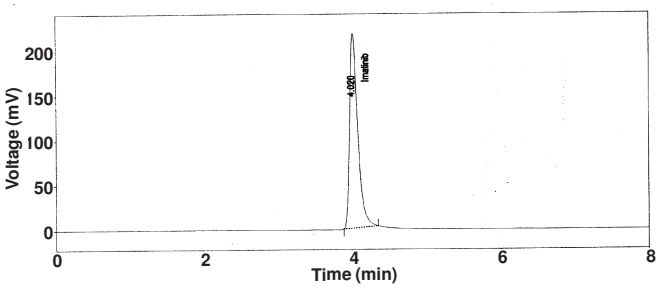

Fig. 3. HPLC chromatogram of Imatinib Mesylate α-form (formulation)

TABLE-3
PRECISION STUDIES

Conc. (µg/mL)	Peak area	% RSD
80	6356.908	0.669626

*Each value is the average of five determinations

TABLE-4
ACCURACY STUDIES

Mixture of pure and formulation (%)	Conc. of formulation (µg/mL)	% of recovery of pure drug	% RSD
80	80	99.9967	0.006014
100	100	99.8276	0.286092
120	120	99.7601	0.117197

*each value is the average of five determinations

TABLE-5
SYSTEM SUITABILITY

S.No.	Parameters	Values
1	Theoretical plates (N)	10125
2	LOD (µg/mL)	0.833
3	LOQ (µg/mL)	2.774

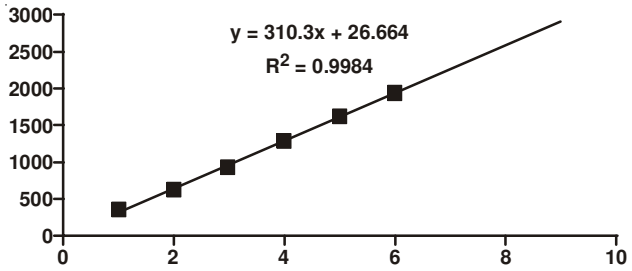

Fig. 4. Linearity of the drug concentration

Conclusion

The results obtained from these studies are well fit into the standard specifications stipulated by the regulatory agencies. The method is able to reproduce the results consistently and the recovery studies of imatinib mesylate α-form are found to be 99.10 %. This indicates that commonly used excipients in pharmaceutical formulation were not interfering in the proposed method. The observation of % C.C less than 2.0 for intra day measurements also indicates high degree of precision. In the present method, we have established a linearity range of 10-60 µg/mL, this linearity range covers all the strengths of imatinib mesylate α-form, hence this can be conveniently used in the pharmaceutical manufacturing and formulation environment.

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