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IJPBS |Volume 1| Issue 2 |APRIL- JUNE |2011

DEVELOPMENT OF RP-HPLC METHOD FOR THE ANALYSIS OF IMATINIB MESYLATE USING PDA DETECTOR AND ITS APPLICATION IN THE EVALUATION OF MARKETED PREPARATION

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Research Article

RECEIVED ON 03-03-2011 **ACCEPTED ON 10-04-2011**

ABSTRACT

A simple, sensitive, isocratic and reproducible reversed phase High Performance Liquid Chromatographic (RP-HPLC) method was developed for the estimation of imatinib mesylate using PDA detector. The system consisted of RP-C18 column and the detection was performed at 260 nm. The mobile phase consist of (4ml tetrabutyl ammonium hydroxide and .01M ammonium dihydrogen orthophosphate (pH adjusted to 3 with orthophosphoric acid) and acetonitrile in ratio 60:40) pumped at room temperature and a flow rate of 1ml/min. Imatinib mesylate was eluted at 2.812min. The mean absolute recoveries of Imatinib mesylate was about 100.1% to 100.3% and the limit of detection (LOD) of imatinib was 10 µg/ml and the limit of quanitation (LOQ) is 20 µg/ml. the method shows good linearity in the range 20 µg/ml to 120 µg/ml (r2>0.995). The intra (n=6) and inter (n=6) day assay variations in the linear range is < 4 .Three marketed products containing imatinib mesylate are analyzed to test the applicability of the new method. The percentage of Imatinib mesylate in marketed preparations studied was found to be 99.58%, 99.92 and 100.85 respective to the label claimed.

KEYWORDS: ***Imatinib mesylate, RP-HPLC, UV-Detection***

Introduction

Imatinib Mesylate is a 2-phenylaminopyrimidine derivative that functions as a specific inhibitor of a number of tyrosine kinase enzymes, There are a large number of tyrosine kinase enzymes in the body, including insulin receptor, Imatinib mesylate is specific for the T.K.domain in Abelson proton cogene, C, kit and PDGF (Platelet Derived Growth factor receptor) Imatinib

mesylate is used in chronic myelogenous leukemia (CML), gastrointestinal stomal tumors (GIS) and a number of other malignancies. In the United States, the food and drug Administration has approved Imatinib as first line treatment for CML (chronic mylgeous leukemia). Imatinib has passed through phase 3 trials for CML, and has been shown to be more effective than the previous standard treatment of a- interferon and cytarabin. Although the long term side effects of Imatinib have not yet been ascertained, researchers suggest that it is generally very well tolerated (ex: liver toxicity was much less than predicted).[1]

Experimental

Pure sample of Imatinib mesylate was gifted by Indu drugs pvt ltd (Hyderabad, A.P.,India). All the solvents were of HPLC and analytical grade purchased merck, Rankem, (Mumbai,India)[2]

Preparation of standard solution

Imatinib mesylate(100mg) was accurately weighed about 50.14mg transfer in to 100ml volumetric flask, dissolved in mobile phase (acetonitrile) phosphate buffer solution (40:60)v/v (P^H 3) (~20ml),and the solution was diluted to volume with the same solvent to get a standard of solution of 0.0501mg. 5ml of this solution was diluted to 25ml volumetric flask to prepare standard II dilution. Take 5ml of standard stock solution to 25ml of volumetric flask and made up to volume with diluent to prepare standard III dilution.[3]

Preparation of test solution

20 IM capsules were weighed and taken average weight of 20capsules, subtracted 20 empty capsule weight from 20 capsule actual weight 25.11mg equivalent of Imatinib mesylate was weighed taken into 50ml volumetric flask volume made upto 50ml with same solvent and allow to sonicate for 20minutes. Test solution was centrifuged at 250rpm for 10minutes using centrifuged tube.[4]

HPLC condition

The samples prepared were analyzed by an isocratic Hplc method. The HPLC analysis was performed on LC-10AT(Shimatzu corporation, Kyoto, Japan) system by injecting 20µl of sample Hamilton Rheodyne syringe (Hamilton Bonaduz AG, Switzerland) into syringe loading sample injector (Model-7725i, Rheodyne LP, CA, USA). The column

used was Luna C_{18}, 5µ, 50x4.6mm i.d.(Phenomenex, USA) The mobile phase consisted of a mixture of acetonitrile:buffer having a composition of (4ml of Tetrabutyl ammonium hydroxide;0.01M Ammonium dihyrogen ortho phosphate and ortho phosphoric acid to maintain pH -3 In 40:60 v/v. The mobile phase was degassed using ultrasonic bath (Sonorex, Bandelin Electronic, Germany). The analysis was performed at ambient temperature with a flow rate of 1ml/min using diode array detector (Shimadzu SPD MIOAUP model Shimadzu corporation, Kyoto, Japan). Detection was carried out at a wavelength of 260nm. The data analysis was performed by class n10 software (Shimadzu Corporation, Kyoto, Japan) [5]

Method validation

The standard solutions were chromatographed for inter and intraday assay variation (n=6).The calibration curves were obtained by plotting the peak area against concentration range. The accuracy of the method was determined using external standard addition. Known amount of standard drugs were added at four different levels, and each determination was carried out in triplicate. The limit of detection (LOD) and quantification were obtained.[6, 7]

Application to the analysis of marketed capsules

Three marketed capsules of Imatinib mesylate were obtained for the analysis using the procedure described above. Ten capsules of each brand were taken and the contents were weighed .Accurately weighed powder of three brands each equivalent 100mg was transferred to three 100 ml volumetric flask, dissolved in mobile phase (~20ml),and shaken for 15 min. The solutions were then diluted to volume with the same solvent, mixed ,and finally filtered through whatmann no.42 filter paper (what middle sex UK).A sample (ml) of each filtrate was serially diluted to get 0.0502mg/ml respectively. These solutions were used for analysis. The analysis was done in triplicate, and the amount of Imatinib mesylate in these marketed preparations was calculated from the calibration curves. [8, 9, 10]

Results and Discussions

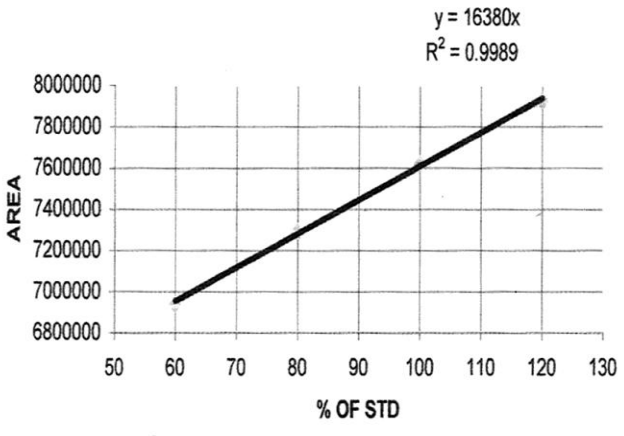
The RT for Imatinib mesylate using this method was found to 2.812min.the chromatograms of Imatinib mesylate using

PDA detector at wavelength 260nm. The percentage recoveries vary from 100.1% to 100.3% and the calibration was linear over a concentration range 0.03mg/ml and 0.06mg/ml (r^2 > 0.998) (Graph-1).the intra (n=6) and inter (n=6) day assay variations in the linear range are less than 1%.

Three pharmaceutical capsules products containing Imatinib mesylate was analyzed to test the applicability of the new method. The percentage of Imatinib in capsule studied as 99.58/100mg and respectively to the claimed value.

GRAPH – 1

LINEARITY OF IMATINIB MESYLATE



Conclusions

The HPLC method developed for the determination of Imatinib mesylate in capsule is accurate, precise, rapid and selective. It can therefore be easily and conveniently use for routine quality control analysis, particularly when large number of samples are encountered. The developed method was found to be specific, as there was no interference of the excipients which is confirmed by the absence of extra peaks.

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