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METHOD DEVELOPMENT AND VALIDATION OF IMATINIB IN CAPSULE DOSAGE FORM BY USING RP-HPLC METHOD

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ABSTRACT

An accurate, Precise and Simple High Performance Liquid Chromatographic method for the estimation of Imatinib in its capsule dosage form has been developed. The method so developed is Reverse Phase High Performance Liquid Chromatographic method using Inertsil C18 ODS column (150mm×4.6mm, 5µ). The method so developed was validated in compliance with the regulatory guidelines by using well developed Analytical method validation tool which comprises with the analytical method validation parameters like Linearity, Accuracy, Method precision, Specificity, System suitability, Robustness and Ruggedness. The results obtained were well within the acceptance criteria.

KEYWORDS

Imatinib, RP-HPLC, Validation and Method development.

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INTRODUCTION[1, 2]

Imatinib is a tyrosine-kinase inhibitor used in the treatment of multiple cancers, most notably Philadelphia chromosome - positive (Ph+) chronic myelogenous leukemia (CML). Like all tyrosine-kinase inhibitors, imatinib works by preventing a tyrosine kinase enzyme, in this case BCR-Abl, from phosphorylating subsequent proteins and initiating the signaling cascade necessary for cancer development, thus preventing the growth of cancer cells and leading to their death by apoptosis. Because the BCR-Abl tyrosine kinase enzyme exists only in cancer cells and not in healthy

cells, imatinib works as a form of targeted therapy only cancer cells are killed through the drug's action. In this regard, imatinib was one of the first cancer therapies to show the potential for such targeted action, and is often cited as a paradigm for research in cancer therapeutics. In the present work, attempts were made to determine Imatinib in capsules by using RP-HPLC. The proposed method is simple and suitable for routine determination of Imatinib in capsules dosage form.

IUPAC name: 4-[(4-methylpiperazin-1-yl) methyl] -*N*-(4-methyl-3-{[4-(pyridin-3-yl) pyrimidin-2-yl] amino} phenyl) benzamide.

MATERIAL AND METHODS[4, 5, 6]

Instruments and chemicals used

Drugs

Imatinib working standard

List of instruments used

A High performance liquid chromatography system with gradient elution capability, a spectro photometric UV detector and an auto sampler (Agilent or equivalent).

Data handling system: EZ Chrome or equivalent.

Analytical Balance.

Sonicator.

HPLC Column: Inertsil ODS C18, 150 x 4.6 mm 5µ or Equivalent.

Preparation of solutions[8, 9]

Preparation of mobile phase

Procedure

Dissolve 0.6825grm of potassium di hydrogen phosphate and 3gms of 1-Hexane sulfonic acid in 500 ml of water. Mixed well filtered and degassed through 0.45µm membrane filter and adjust the pH to 3.0 with formic acid. Methanol (HPLC grade) of 600 mL was taken and 400 mL of the above prepared buffer was added to it and finally this solution was filtered through 0.45µm membrane filter followed by degassing.

Preparation of standard stock solution

Procedure

Weigh accurately about 60.0 mg of Imatinib mesylate working standard into 50ml volumetric flask. Add about 10ml of mobile phase and sonicate for 5mins and dilute to volume with mobile phase. Transfer 5ml of above solution into 50ml volumetric flask and dilute to volume with mobile phase.

Preparation of sample stock solution

Procedure

Open, the contents of 20 capsules and mix. Transfer an accurately weighed portion of the powder equivalent to 100mg of Imatinib into 100ml volumetric flask. Add about 50ml of mobile phase, sonicate for 10mins with occasional stirring and dilute to volume with mobile phase, mix and filter. Transfer 5ml of this solution into 50ml volumetric flask and dilute to volume with mobile phase.

METHOD VALIDATION[10]

Definition: It is defined as establishing documentation evidence, which provides a high degree of assurance that specific process, will consistently produce meeting its predetermined specification and quality attributes.

System Suitability

Definition: It can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision.

Procedure: Weigh and transfer accurately 60 mg of Imatinib mesylate working standard in to a 50 ml volumetric flask, add about 30 ml of diluent, sonicate for 5 minutes and make up to the volume with diluent, dilute 5mL of the resultant solution to 100mL with diluent. 5mL of the resultant solution was further diluted to 50mL with diluent. The acceptance criteria were ± 2% for percent coefficient variation (CV %) for peak area and retention time for Imatinib.

Chromatogram for the above discussed system suitability solutions were recorded and results are listed. The results are shown in Table No.2.

Specificity

Definition: The ability to assess unequivocally the analyte in the presence of components that may be expected to present, such as impurities, degradation products and matrix components, etc.

The specificity of the method is performed by separately injecting the blank, Imatinib sample and

placebo. The interference observed (if any) at the retention times of each analyte in all the chromatograms is evaluated. Chromatograms for the above discussed specificity solutions were recorded.

Linearity

Definition: The linearity of an analytical method is its ability to obtain test results which are directly or indirectly (by a well-defined mathematical transformation) proportional to the concentration of the analyte in a sample within the given range".

Procedure: The calibration curves were constructed with five concentrations ranging from 60 to 150µg/mL for both Imatinib. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Preparation of solution-1: Transfer 3 ml of stock solution in to a 50 ml volumetric flask, add 35 ml of diluent, mix well and make up the volume with diluents to get a concentration of 60µg/ml.

Preparation of solution-2: Transfer 4 ml of stock solution in to a 50 ml volumetric flask, add 35 ml of diluent, mix well and make up the volume with diluents to get a concentration of 80µg/ml.

Preparation of solution-3: Transfer 5 ml of stock solution in to a 50 ml volumetric flask, add 35 ml of diluent, mix well and make up the volume with diluents to get a concentration of 100 µg/ml.

Preparation of solution-4: Transfer 6 ml of stock solution in to a 50 ml volumetric flask, add 35 ml of diluent, mix well and make up the volume with diluents to get a concentration of 120 µg/ml

Preparation of solution-5: Transfer 7.5 ml of stock solution in to a 50 ml volumetric flask, add 35 ml of diluent, mix well and make up the volume with diluents to get a concentration of 150µg/ml Chromatograms for the above discussed linearity solutions were recorded and results are listed.

Accuracy and precision

Definition: The accuracy of a measurement is defined as the closeness of the measured value to the true value .A sample (whose "true value" is known) is analyzed and the measured value should ideally be identical to the true value with high accuracy.

Precision can be defined as "the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample". The results are shown in Table No.4.

Preparation of LQC sample: Transfer accurately 2.5mL of Imatinib stock solution and 115 mg of placebo in to a 100 ml volumetric, flask add 50 ml of diluent sonicate for 5 minutes with occasional stirring and make up the volume with diluent and filter. The concentration is 80µg/ml.

Preparation of MQC sample: Transfer accurately 5.0 mL of Imatinib stock solution and 115 mg of placebo in to a 100 ml volumetric flask, add 50 ml of diluent, sonicate for 5 minutes with occasional stirring and make up to the volume with diluent and filter. Prepare the samples in triplicate. The concentration is 100µg/ml.

Preparation of HQC sample: Transfer accurately 7.5 mL of Imatinib stock solution and 115 mg of placebo in to a 100 ml volumetric flask, add 50 ml of diluent, sonicate for 5 minutes with occasional stirring and make up to the volume with diluent and filter. Prepare the samples in triplicate. The concentration is 120µg/ml. Accuracy of the assay method was determined for both intra-day and inter-day variations using the triplicate analysis of QC samples. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time that was evaluated by assaying the QC samples during same day. Intermediate precision was assessed by comparing assays on different days (3days).

Chromatograms for the above discussed linearity solutions were recorded and results are listed.

Ruggedness

Definition: The Ruggedness was determined by using the data obtained by the analysis performed by two different analysts.

Procedure: Each analyst prepared 3 MQC samples of the same batch and the results obtained.

Chromatograms were recorded and results listed**.** The results are shown in Table No.5.

Definition: It is a measure of method's ability to in method parameters and provides an indication of its reliability during normal usage.

Procedure

The MQC sample solution prepared was analysed under different chromatographic conditions stated below.

Change in flow rate

Flow rate – 0.9 mL/min

Flow rate – 1.1mL/min

remain unaffected by small but deliberate variations

Change in mobile phase

MeOH: buffer (65:35 % v/v)

MeOH: buffer (55:45 % v/v)

RESULTS AND DISCUSSION

System suitability

The % CV of peak area and retention time for Imatinib was within 2 % indicating the suitability of the system.

Table No.1: List of chemicals and solvents used

S.No	Chemicals and Solvents
1	Methanol (HPLC Grade)
2	Potassium di hydrogen phosphate
3	Milli Q Water
4	1-Hexane sulfonic acid
5	Formic acid

Table No.2: System Suitability

System suitability parameters	Imatinib
%RSD for six replicate injections of standard	0.26
Tailing factor	1.15
Theoretical plates	3219
Resolution	-

Table No.3: Linearity

S.No	Target Concentration (%)	Concentration (mg/ml)	Area
1	60	0.06083	4955588
2	80	0.08111	6427448
3	100	0.10139	8097774
4	120	0.12166	9968417
5	150	0.15208	12212898
Correlation Coefficient [R]			0.999
Slope			80830144.39
Intercept			26485.54

Table No.4: Accuracy and Precision of Imatinib data

S.No	Sample	Experimental Concentration (mg/ml)	Theoretical Concentration (mg/ml)	Recovery (%)
1	80%	0.00252	0.0025	99.4
2	80%	0.00254	0.0025	100.4
3	80%	0.00252	0.0025	99.4
4	100%	0.00512	0.0051	101.0
5	100%	0.00520	0.0051	102.6
6	100%	0.00499	0.0051	98.4
7	120%	0.00783	0.0076	103.0
8	120%	0.00762	0.0076	100.2
9	120%	0.00779	0.0076	102.4
	Mean			100.75
	Standard deviation			4.74
	% RSD			4.70

The % Recovery should be in between 85.0 to 115.0, The results indicate that the recovery of Imatinib is good within the specified range.

Table No.5: Ruggedness data

	Drug name	Rt	Tailing factor	Theoretical plates	Resolution
Analyst 1	Imatinib	7.736	1.30	1818	-
Analyst 2	Imatinib	7.734	1.28	1894	-

Table No.6: Effect of mobile phase flow (± 10% of Actual flow)

	Actual flow (1.0 ml/min)	**10% Less flow (0.9 ml/min)**	**10% Excess flow (1.1 ml/min)**
Impurity (% w/w)	0.254	0.244	0.258
Retention time (min)	7.3	7.82	5.80
% RSD of Reference solution	0.43	0.55	1.63

There is no significant effect on the result by doing small changes in the mobile phase flow

Table No.7: Effect of wavelength (± 2 nm)

	Actual (264nm)	**262 nm**	**266 nm**
Impurity (% w/w)	0.270	0.276	0.224
Retention time (min)	7.42	7.20	7.00
% RSD of Reference solution	0.32	0.86	1.28

There is no significant effect on the result by doing small changes in the wavelength.

Table No.8: Effect of changes in mobile phase composition (±5% Organic solvent)

	Actual MP	**5% Excess Organic solvent**	**5% Less Organic solvent**
Impurity (% w/w)	0.254	0.247	0.230
Retention time (min)	7.3	6.55	8.10
% RSD of Reference solution	0.43	2.16	0.50

There is no significant effect on the result by doing small changes in the organic solvent composition in mobile phase.

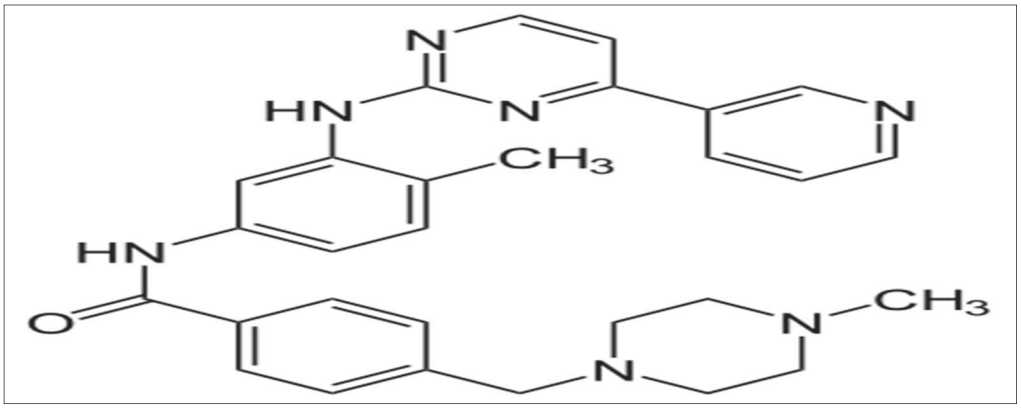

Figure No.1: Chemical Structure of Imatinib

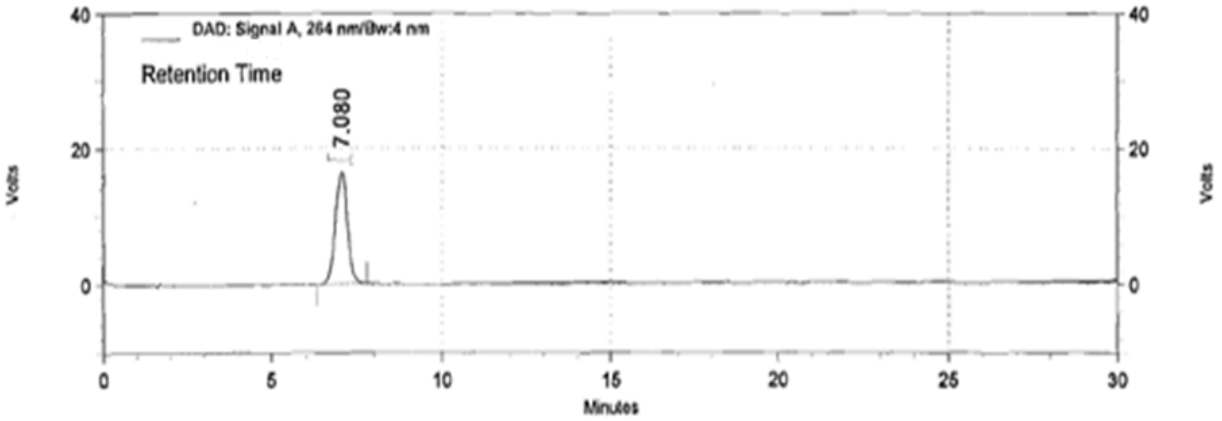

Figure No.2: Chromatogram for system suitability

CONCLUSION

A simple and reproducible HPLC procedure was developed and validated as per ICH guidelines for the estimation of Imatinib.

Quantitative estimation of Imatinib was estimated by RP-HPLC using MeOH: Pot. DiHyd Phosphate buffer (60:40 % v/v) as a mobile phase and Inertsil column (150mm×4.6mm, 5µ) as a stationary phase and the peaks were observed at 264nm which was selected as a wavelength for quantitative estimation. After development of the method it was validated for specificity, system suitability, accuracy, linearity, precision, ruggedness and robustness. The value of theoretical plates, tailing factor, retention time and peak area was found to be within limits, hence it is concluded that the system is suitable to perform assay. The method was found to be specific because it did not show any interference with placebo and blank.

The linearity studies were performed for the standard and found to be linear. From the linearity studies, the specified range was found to be 60 µg/mL to150 µg/mL of the target concentration of Imatinib. The precision was checked and found to be within limits, hence the method is precise. The accuracy has been determined from LQC to HQC and the prescribed limits for recovery are 98%-102%. From accuracy studies, % recovery was calculated and found to be within limits. The ruggedness of the method was checked on different analysts and drug was able to give same results which indicate that the method is rugged. The robustness of the method was checked by changing flow rate, wavelength and mobile phase composition and drug was able to give system suitability parameters within limit, which indicates that the method is robust.

Therefore it was concluded that the proposed method can be used for routine analysis of Imatinib Capsule dosage forms.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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