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Determination of Potential Genotoxic Impurities in *Imatinib Mesylate* by RP-HPLC Method

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ABSTRACT : A gradient reversed-phase HPLC method with PDA detector has been developed for the purity evaluation of Imatinib Mesylate in bulk drug. The impurities are (2-methyl-5-aminophenyl)-4-(3-pyridyl)-2-pyrimidine amine (*i.e.* Imp-A) and N-[4-methyl-3-(4-methyl-3-yl-pyrimidin-2-ylamino)-phenyl]-4-chloromethyl benzamide (*i.e.* Imp-B). The analysis was performed using inertsil ODS 3V column (150 × 4.6 mm, 5µ) as a stationary phase with column oven temperature 35°C and UV detection at 268 nm. The separation was achieved using gradient program of buffer (A Buffer used was of 0.1% Triethyl amine in water and pH adjusted to 2.9 with glacial acetic acid) and mixture of methanol and Acetonitrile. The method was optimized based on the peak shapes and resolution of Imp-A and Imp-B. The method was validated as per International Conference of Harmonization (ICH) guidelines in terms of limit of detection (LOD), Limit of quantitation (LOQ), linearity, precision, accuracy, specificity, robustness and solution stability. The LOD and LOQ values were found to be 0.024 µg/ml and 0.08 µg/ml, respectively. The sample concentration were injected was 10 mg/ml. The method is linear within the range of 0.08-0.3 µg/ml for both the Impurities.

Keywords : Imatinib Mesylate; Imp-A; Imp-B; Cancer drugs; Genotoxic Impurity.

INTRODUCTION

Drug substances (DS) process development and Drug product (DP) formulation development are two major areas of the drug development process. Impurities/degradants can be generated in either of the processes, from DS degradation or DS-Excipient interaction. These Impurities either non-genotoxic or genotoxic in nature. Regardless, they are regulated by food and drug Administrator (FDA)/ International conferences on Harmonization (ICH) guidelines. Routine impurity analysis in pharmaceuticals requires identification at levels of 0.05 percent to 0.2 percent depending on the daily dose. However, genotoxic impurities can be much harder to detect due to their presence at low ppm levels. This review concentrates on the regulations and analytical technologies used to detect and quantitate impurities (genotoxic and non-genotoxic) in pharmaceuticals [1].

Imatinib is a drug used to treat certain types of cancer. It is used in treating chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs) and some other diseases [2]. Imatinib is protein tyrosine kinase (PKT) inhibitor which potentially inhibits Abelson (Abl) tyrosine kinase in Vitro studies [3-5]. In this work we demonstrate the practical example for the analytical control of two genotoxic impurites in Imatinib mesylate. These impurities were observed to be process impurities. From the literature it was found out that these impurites are genotoxic [6-14]. The method is based on High performance liquid chromatography (HPLC) for determination of N-(2-methyl-5-aminophenyl)-4-(3-pyridyl)-2-pyrimidine amine (*i.e.* Imp-A) and N-[4-methyl-3-(4-methyl-3-yl-pyrimidin-2-ylamino)-phenyl] -4- chloromethyl benzamide (*i.e.* Imp-B). The method was validated as per International conference of Harmonization (ICH) guidelines in terms of limit of specificity, linearity accuracy, detection (LOD), Limit of quantitation (LOQ), precision and solution stability [15].

MATERIAL AND METHODS

A. Drug and reagents

Pure Imatinib mesylate was obtained as gratis sample from Cipla Ltd. Research Laboratories (Mumbai, India). Analytical reagent (AR) grade triethyl amine was purchased from Fluka (Banglore, India), acetic acid from Merck (Mumbai, India) Acetonitrile from sigma Aldrich (Mumbai, India) and Methanol (HPLC grade) from Lab-chem, (Goa, India). Water for HPLC studies was obtained from milipore water purifying system.

B. Apparatus and equipment

LC was carried out on Agilent HPLC (1200 series, Germany) with photodiode array detector. The output signal was monitored and processed using Chromeleon software (ver number 6.80SR10). In all the studies, separations were achieved on a Inertsil ODS 3V column (150 mm × 4.6 mm i.d., particle size 5 µm) procured from LCGC (Banglore, INDIA).

A pH/Ion analyzer (Labindia, made in) was used to check and adjust the pH of buffer solutions. Other small equipment were PCI sonicator (22L500/CC/DTC made in), precision analytical balance (MX5, Mettler Toledo, Schwerzenbach, Switzerland).

C. Preparation of mobile phase

The Solution A was prepared by using 0.1% Triethyl amine in water and pH adjusted to 2.9 with glacial acetic acid. Solution B contains methanol and acetonitrile in the ration of 10 : 90 (v/v).

D. Chromatographic conditions

The numbers of column such as waters symmetry C18 (150 × 4.6 mm, 5.0µm), YMC packpro C18 (150 × 4.6 mm, 5.0 µm) phenomenex luna C18(2)(150 × 4.6 mm, 5.0µm) were used during method development. The separation was achieved using gradient program of solution A (*i.e.* Solution A used Contains 0.1% Triethyl amine in water and pH adjusted to 2.9 with glacial acetic acid): and Solution B (Mixture of Acetonitrile and methanol in the ration of 10 : 90 v/v) the flow rate was set at 1.5 ml/min and column was maintained at 35°C. The injection volume was set 20 µl and detector was set at a wavelength of 268 nm. For gradient program (Table 1).

Table 1 : Gradieant Program.

Time	*% Solution A*	*% Solution B*
0.01	90	10
10.0	90	10
20.0	50	50
28.0	50	50
32.0	10	90
38.0	10	90
38.1	90	10
45.0	90	10

Table 2 : Accuracy Results of Impurity-A.

Added (µg)	*Recovered (µg)*	*% Recovery*	*% RSD*
	9.73	108.13	
9.0	9.78	108.75	2.64
	10.21	113.44	
	12.10	110.0	
11.0	11.29	102.6	3.61
	11.90	108.2	
	23.84	108.35	
22.0	24.17	109.85	0.85
	23.79	108.15	
	36.17	109.62	
33.0	35.19	106.60	2.50
	36.98	112.07	

Table 3 : Accuracy Results of Impurity-B.

Added (µg)	*Recovered (µg)*	*% Recovery*	*% RSD*
	7.80	97.50	
8.0	7.32	91.56	3.35
	7.72	96.56	
	8.19	91.00	
9.0	8.24	91.56	0.88
	8.33	92.60	
	17.83	93.85	
19.0	17.79	93.65	1.21
	17.44	91.80	
	24.79	91.80	
27.0	24.72	91.56	1.22
	24.24	89.76	

Table 4 : Lod and LOQ Results of Impurities.

Compound	*LOD (ng/ml)*	*% RSD*	*LOQ (ng/ml)*	*% RSD*
Impurity-A	2.4	9.7	8.0	4.39
Impurity-B	2.4	2.3	8.0	1.7

Table 5 : Results Obtained Form Three Batches of Imatinib Mesylate.

Compound	*Impurity-A*	*Impurity-B*
B. No. A	Not Detected	Not Detected
B. No. B	Not Detected	Not Detected
B. No. C	Not Detected	Not Detected

E. Preparation of sample during method development

The diluent was selected for dissolving imatinib and its impurities was mixture of Solution A and Solution B (in ration of 50 : 50 v/v). Stock solution of imatinib impurities were prepared in diluent having concentration of 0.2mg/ml. Five thousand micro liter of impurities stock solution were transferred to 100ml volumetric flask and diluted up the volume with diluent. Further transferred 2000µl this solution to 100ml volumetric flask and diluted up the volume with diluent. Imatinib mesylate sample solution were prepared in the concentration 10mg/ml. The concentration of imatinib impurities was 0.002% was injected.

F. Preparation of sample for validation

Stock solution of Imatinib impurities and imatinib were prepared in diluent. These stock solutions of impurities were further diluted with diluent to get the required concentration for validation studies.

RESULTS AND DISCUSSION

A. Method development and column selection

Chemical structure of imatinib mesylate and its related impurities are shown in (Fig. 1). The impurities are labeled as Impurity-A and Impurity-B is the precursor for imatinib. The production batch sample of imatinib mesylate which was selected for validation studies.

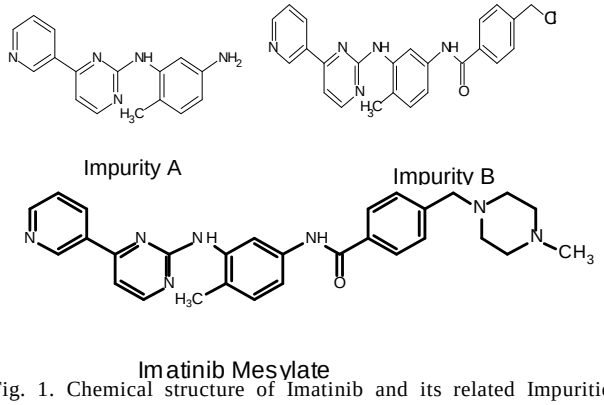

Fig. 1. Chemical structure of Imatinib and its related Impurities.

Different mobile phase and stationary phases were employed to developed a suitable LC method for the quantitative determination of impurities present in imatinib mesylate. A number of column containing various packing materials of ODS supplied by different manufacturers and different mobile phase composition were tried to get good peak shapes and selectivity for the impurities present in imatinib. Poor peak shape and resolution was observed when phosphate buffer with different composition of acetonitrile was employed. In the next approach mixture of Phosphate buffer, acetonitrile and methanol were used in the ration of (50 : 25 : 25 v/v/v) using waters symmetry C18 (150 × 4.6 mm, 5.0 µm) column. Under these condition Impurity-A and impurity-B eluted in close proximity to imatinib's other unknown impurities. With decrease of methanol and Acetonitrile content and increased content of phosphate buffer, no significant changes were observed.

In the another attempt using phenomenex luna C18(2) (150 × 4.6 mm, 5.0 µm) and mobile phase consisting of mixture of 0.1% triethylamine in water: Acetonitrile and Methanol (80 : 10 : 10 v/v/v) Imatinib eluted at 12 min good separation, however peak shape of impurity-A was not good.

By using another attempt with mixture of mobile phase 0.1% Triethylamine, Acetonitrile and methanol (75 : 15 : 10 v/v/v/) and column Inertsil ODS, imatinib impurity-A eluted in close proximity to imatinib other unknown impurities.

The separation was achieved using gradient program of Buffer (A Buffer used was of 0.1% Triethyl amine in water and pH adjusted to 2.9 with glacial acetic acid): Acetonitrile: Methanol. The method was optimized based on the peak shapes and resolution of Imp-A and Imp-B (Fig. 2).

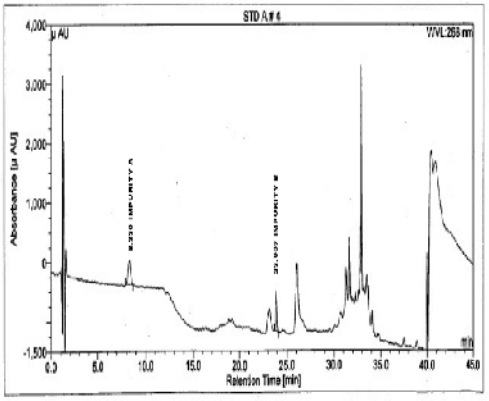

Fig. 2. Typical chromatogram of Imatinib Impurity A and Impurity B in Inertsil ODS3V (150 × 4.6 mm, 5.0 µm) column, Mobile phase consisting of 0.1% Triethyl amine in water and pH adjusted to 2.9 with glacial acetic acid and mixture of Acetonitrile: Methanol (80 : 20) with gradient composition and flow rate of 1.0 ml/min and UV detection at 268 nm.

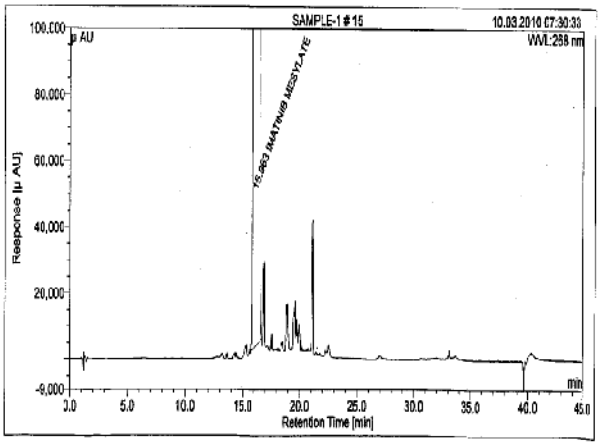

Fig. 3. Typical Sample chromatogram of Imatinib Mesylate.

B. Method validation

(*a*) *Specificity*

Specificity of the method is its ability to detect and separate all the impurities present in the drug. Specificity of the method is demonstrated in terms of spectral as well as peak purity data of the drug and impurities present in drug. Peak passed the peak purity test.

(*b*) *Linearity*

Linearity of the method was checked by preparing solutions at six concentration levels of 0.0008 (Level 1), 0.001 (Level 2), 0.0015 (Level 3), 0.002 (Level 4), 0.0025 (Level 5) and 0.003 (Level 6) % for Impurity-A and Impurity-B. Level 1 and level 6 was injected six times were as level 2, level 3, level 4 and level 5 was injected two times. The mean responses recorded for each impurity were plotted against concentration. The correlation coefficient for impurity-A and impurity-B was found to be 0.99904 and 0.99980 respectively, which indicates good linearity.

(*c*) *Accuracy*

Imatinib solution was spiked with each impurity solution at different concentration at 0.0008, 0.001, 0.002 and 0.003%

of analyte concentration 10mg/ml. Each spiked solution was prepared in triplicate and injected. The recovery percentage and % RSD were calculated for each impurity. Recovery of Impurity-A and Impurity-B ranged from 108.15-109.85% and 91.80-93.85% respectively. The results are shown in Table 2-3, respectively. The acceptance criteria for recovery of an impurity at a concentration level of 0.002% is between 85 and 115%.

(*d*) *Limit of detection*

The sensitivity for detection can be demonstrated by determining the limit of detection (LOD). A signal to noise (S/N) ratio between 3 to 10 is generally considered to be acceptable for estimating detection limit. S/N ratios of individual peak were determined at different concentration at estimate LOD and respective %RSD was calculated for replicate injection (n = 3). The LOD was found to be 0.00024% (2.4 ng/ml) for impurity-A and for impurity-B 0.00024% (2.4 ng/ml). The results are shown in the Table 4.

(*e*) *Limit of quantification*

The quantification limit is the lowest concentration of a substance that can be quantified with acceptable precision and accuracy. A typical S/N ratio of 10-30 is generally considered to be acceptable for estimating the limit of quantification. S/N rations of individual peaks were determined at different concentration to estimate limit of quantitation (LOQ) and respective % RSD was calculated for replicate injection (n = 6). The LOQ was determined to be 0.0008% (8ng/ml) for both Impurity-A and Impurity-B. The results are shown in Table 4.

(*f*) *System and method precision*

The system for two impurities in imatinib was checked for repeatability. The sample was prepared by spiking imtinib with the impurity at a concentration of 0.002% of target analyte concentration and injected six times. The % RSD was found to be less than 5.0% for system precision.

To determine the method precision six independent solution were prepared by spiking imatinib with the impurities at a concentration of 0.002% with respect to target analyte concentration. Each solution was injected once. The variation in the results for the two impurities were expressed in terms of % RSD. The values calculated were found to be below 15.0% RSD for impurities, indicating satisfactory method precision.

(*g*) *Stability in analytical solution*

A solution of Imatinib containing impurities was prepared and kept at room temperature. This solution was injected at intervals of 0, 4, 8, 12, 16, 20 and 24hr. Area of all the impurities were nearly identical to that obtained at 0*h* and additional peaks were not observed which indicate solution stability.

Sample preparation of Imatinib for Routine Analysis.

Weighed 100mg of imatinib mesylate sample in 10ml volumetric flask, dissolved in diluents and dilute up the volume with diluents. Injected this solution into HPLC to determine the amount of impurities present in the sample. Three different batches of imatinib mesylate was analyzed under developed condition and presented the results in Table 5. The chromatogram obtained after the analysis was shown in (Fig. 3). Some of the small peaks observed in the chromatogram are due to impurities present in the batch sample, which were analyzed by another method with limit of 0.1%

CONCLUSION

The proposed LC method is selective for the quantification of Genotoxic impurity-A and Impurity-B present in Imatinib. The method is capable of detecting two process intermediates.

Hence this method is useful for the detection of Genotoxic impurities present in Imatinib mesylate.

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