Determination of *Genotoxic* Impurities in *Nilotinib using* LC-MS Method

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ABSTRACT:

A validated HPLC method was developed for the determination of Nilotinib (Nob) in pharmaceutical formulation. Isocratic elution at a flow rate of 1.0ml/min was employed on Zorbax SB C18 5µm × 4.6mm, 150mm, or similar is used for this chromatography analysis and the column temperature is maintained as ambient. Mobile phase was a mixture of 2.00ml of acetic acid in 2000ml of water as mobile phase A and Acetonitrile 100% as mobile phase B was used. Rate of flow is identified at 1.3ml/min. a 50.0µl sample was injected. The run time is 20 minutes to Sample, blank, system suitability, and sensitivity solution. For the diluted standard solution, it is 60 minutes. The retention time was 1.30minutes. The% R.S.D Nob is identified. The mean percentage recovery for Nob is found within the specification limit. Due to this reason this proposed LC-MS method is successfully adopted to routine quality control analysis in formulations.

KEYWORDS: Genotoxic impurities, Nilotinib, Liquid Chromatography-Mass Spectrometry (LC-MS) method; Validation and limit of quantitation.

1. INTRODUCTION:

Nilotinib (Nob), marketed under variety name as "Tasigna", It is a durg utilized for the treat of CML which consists of Philadelphia chromosome.[1] This drug is used both the cases like chronic phase as well as in accelerated and chronic phase CML. Nob is acting as Bcr-Abl tyrosine kinase inhibitor by signaling within cell which can able to give the cancer.[2] Nob brings a black box warning in the country named US to conceivable complications related to the heart.[3,4] The interaction of Nob by OATP1B1 and OATP1B3 should changes its hepatic complexation also should point to transporter mediated interactions of drug to drug.[5] Nob is acting as an inhibitor to OATP-1B1 transporter but not to OATP-1B3.[6] V D N kumar abbaraju[7] developed RP - HPLC Method to determine darunavir in bulk and pharmaceutical formulation by using waters Xterra RP-18(150*4.6mm, 3.5µm) column. pH is 3.5.

Mobile phase is 10mM ammonium formate. 20µl is injected volume. 1.00ml/min is rate of flow. 250nm is wavelength. Rt is 4.37, 7.40, 8.96, 10.21 and 10.87min. Mohammed Sameera Bhanu et.al.,[8] developed Bilastine and Monteleukast Sodium. Column is used as Zodiac sil RP C18 column (100mm × 4.6mm, 3µm). Mobile phase as 75% phosphate Buffer as 25v/v. pH is 5.2. Rate of flow as 1.00ml/min. Wavelength as 272nm. Rt for these two drugs are 3.668min. and 2.746 min. Linearity ranges for the both the drugs 20-100µg/ml and5-25µg/ml with correlation coefficient as 0.999. Gurumurthy. et,al,[9] developed Eprosartan mesylate and hydrochlorothiazide. Analysis is performed on Agilent system C18 (150mm x 4.6mm, 5m) column. Mobile phase is 0.1% orthophosphoric acid 60v/v and Acetonitrile as 40v/v. 1.0ml/min is the flow rate. 240nm is the wavelength. Calibrations curves are constructed for these two drugs varies as 80-3200ng/ml and 8.5-340ng/ml. Precision, Mean recovery are calculated. S. Sangeetha et.al.,[10] are used C-18 BEH _ 1.7µm x 2.1 x 50mm. 0.8ml/min is the flow rate measured. 278nm is the wavelength. Mobile phase is acetonitrile 50v/v and Buffer 50v/v. pH is 3.5. Run time as 4min. The percentage purity along with Rt are 99.72 and 99.30 and 1.193 and 1.827. Different authors[11-17] are selected different drugs single/combination drugs and concluded that their methods are precise, accurate and results are in acceptable limits.

2. EXPERIMENTAL:

Zorbax SB C18 5µm (4.6mm x 150mm) or equivalent is utilized for this analysis. The mobile Phase – A is 2.0ml Acetic acid in to 1000ml standard flask maintained up to 6 min. at 4°C. pH is adjusted to 3 by Phosphoric acid. The mobile phase – B is prepared by using Acetonitrile as 100%. Diluent 1 is 80:20v/v of acetonitrile and water. And diluent 2 is 300ml of N, N Di methyl acetamide and 200ml of diluent – 1. Impurity – A Stock solution is prepared by taking equivalent to 20.0mg of Impurity – A {3-(Trifluoromethyl)-5-4(methyl 1H-imidazol-1-yl)} benzene amine hydrochloride standard into 100.00ml of standard flask and diluted by diluent -1. Impurity – B Stock solution is prepared by taking 20.0mg of impurity – B (Methyl 3 amino 4-methylbenzoate) standard into 100.00ml standard flask, diluted by Acetonitrile. Impurity – C Stock solution is prepared by taking 20.0mg of impurity – C (3 amino 4-methylbenzoic acid) standard into 100.00ml

standard flask, diluted acetonitrile. Test and standard solutions are prepared by taking 0.750ml Impurity – A stock, 0.50ml of Impurity – B and C into 500.00ml of standard flask, diluted by diluent – 2. Later, dilute 5.00ml of this in 50.00ml standard flask, diluted by diluent – 2. Test solution is prepared by taking 100.00mg of Nb test sample into 10.00ml of standard flask, added 6.00ml of N, N-Dimethyl Acetamide, diluted by diluent – 1. Retention times for these Impurities are 60.00min., 11.00min., and 4.00min. respectively.

3. METHOD DEVELOPMENT:

To this case disparate parameters were studied as well as considered to this Nob in tablet dosage form of strength as 15.00mg. To identify rate of flow, mobile phase is changed from 0.50mL/min to 1.50mL/min to identify very good separation. Finally, noted from the experiment, the 1.30mL/min rate of flow is most suitable for eluting the analyte.

4. VALIDATION OF PROPOSED METHOD FURTHERMORE REQUIREMENTS:

Specificity:

All these solutions were analyzed as per LC-MS/MS process described in the table 1. There is no intrusion noted at the retention time of Impurity's – A, B and C, hence this method is specific for the determination of Impurity – A, B and C.

Table: 1 Results for Specificity

Solution	Acceptance Limit	Results	
		Impurity	% RSD
System Suitability	% RSD for the area of each impurity from six replace injections from standard solution NMT 15.0	Impurity - A	0.9
		Impurity - B	2.1
		Impurity - C	2.4
Specificity	No interference should be noted at retention time of Impurity – A, B as well as C in the blank	There is no interference noted at this RT of Impurity – A, B and C in the blank sample.	

Linearity and range:

From the figures it is finally concluded that Impurity – A, B and C in specified strength ranges are more satisfactory, by a correlation coefficient which is greater than to the value 0.99 and intercept with respect to 100% standard response is less than ±10.0. Results obtained are shown in the table 2 and in the figures are shown in Figure 1 to 6.

Table 2: Linearity results for Impurity A, B and C

Level	Impurity - A		Impurity - B		Impurity - C	
	Average Area	Strength in µg/mL	Average Area	Strength in µg/mL	Average Area	Strength in µg/mL
LOQ	265627	7.407	113624	5.122	33798	5.072
50%	519486	14.813	221117	10.245	67114	10.144
100%	985010	29.626	444374	20.489	13787	20.287
120%	1199528	35.552	545034	24.587	164710	24.345
150%	1461381	44.440	677284	30.734	207177	30.431

Fig – 1: Linearity for impurity – A

Fig – 2: Linearity 100% for Impurity – A

Fig – 3: Linearity 50% for Impurity – B

Fig – 4: Linearity 120% for Impurity – B

Fig – 5: Linearity 50% for Impurity – C

Fig – 6: Linearity 120% for Impurity – C

Figure: 1 to 6 Linearity curves for Different impurities

Accuracy:

Percentage recovery results obtained for Impurity – A, B and C content were listed in the table 3.The percentage individual recovery and mean recovery values obtained were in the range of 93.38% - 99.75% and 93.8-97.4% for impurity – A, 93.22 - 103.81% and 97.4% - 101.6% for impurity – B and 91.06% - 101.22% and 94.2% -100.8% for impurity – C respectively which were within the specified acceptance criteria set in the validation protocol. The results are obtained are represented in table 3, related chromatograms are denoted in the figure 7 to 10.

Table: 3 % Recovery for Impurity – A, B and C

	Impurity - A	Impurity - B	Impurity - C
Level(specification)	Recovery (%)		
LOQ	97.4	97.4	94.2
100%	93.8	99.3	96.1
150%	94.8	101.6	100.8

Fig – 7: Accuracy at 150% for Impurity – B

Fig – 8: Accuracy at 150% for Impurity – C

Fig – 9: Accuracy at 100% for Impurity – B

Fig – 10: Accuracy at 150% for Impurity – A

Figure 7 to 10: Accuracy curves for various impurities

Table 4: Comparison results of method precision and Intermediate Precision Limit of Detection (LOD):

Sample (100% specification limit)	Impurity – A in ppm		Impurity – B in ppm		Impurity - C in ppm	
	Metho d Precis ion	Intermedi ate Precision	Method Precision	Intermediate Precision	Method Precision	Intermediate Precision
Spiked - 1	3.229	3.779	1.955	2.093	1.942	2.289
Spiked - 2	3.260	3.923	2.077	2.210	1.968	2.210
Spiked - 3	3.238	3.818	2.006	2.127	1.944	2.180
Spiked - 4	3.221	3.873	1.996	2.178	1.955	2.162
Spiked - 5	3.202	3.875	2.004	2.057	1.952	2.266
Spiked – 6	3.174	3.935	1.985	2.046	1.898	2.143
Average	3.544		2.504		2.076	
Std.dev.	0.34060		0.06740		0.14490	
% RSD(12 determination should not be more than 15.0)	9.6		3.3		7.0	

Method Precision and Intermediate Precision:

This parameter is driven by analyzing an unspiked test sample and six spiked test preparations, where Impurity – A, B and C spiked with Nob drug substance at 100% of the specification limit. The long term %RSD to method precision and intermediate precision from 12 spiked sample preparations of Nob drug substance obtained for Impurity's – A, B and C content are within limit defined in protocol. The results are obtained in the Table 4.

The LOD value attained was about 0.370ppm, 0.256ppm and 0.254 with S/N ratio more than 3.0 for Impurity – A, B and C. The results are tabulated in table 5.

Table 5: Limit of Detection

Impurity	LOD w.r.t test conc.(ppm)	Concentration (µg/mL)	S/N ratio (more than 3.0)
Impurity – A	0.370	3.703	22.4
Impurity – B	0.256	2.561	6.4
Impurity – C	0.254	2.536	4.7

Limit of Quantification (LOQ):

LOQ value obtained was about 0.741ppm, 0.512ppm and 0.507ppm with S/N ratio more than 10.0 for Impurity – A, B and C with respect to test concentration of Nob test sample. The results are obtained in the table 6.

Table 6: Limit of Quantification

LOQ w.r.t test conc. (ppm)	Impurity	Concentration (µg/mL)	S/N ratio(more than 10.0)
0.741	Impurity – A	7.407	46.7
0.512	Impurity – B	5.122	14.2
0.507	Impurity – C	5.072	12.9

Precision at LOQ level:

The precision at LOQ was performed by analyzing a total six replicate injections prepared at LOQ strength levels for Impurity – A, B and C. The %RSD was evaluated for peak responses of Impurity – A, B and C. The %RSD for the peaks area of Impurity – A, B and C at LOQ level is less than 15.0indicates that the method is considerably precise to detect the lower levels of respective impurity. The results are tabulated in Table 7.

Table: 7 Summary peak areas for precision at LOQ level

Injection No.	Area		
	Impurity - A	Impurity - B	Impurity - C
1	265642	115418	33.903
2	265752	113742	34278
3	265486	111711	33212
4	265745	114214	33244
5	267560	110393	33196
6	258884	112383	34263

Average	261815	112977	33683
Std.dev.	3016.3869	1827.9236	527.1840
% RSD	1.1	1.6	1.6

5. CONCLUSION:

In order to optimize the mobile phase, different measurements of HPLC Grade mobile phase – A and mobile phase B were tested. Here the composition of the mobile phase B author is used Acetonitrile as 100% culminating in a peak with very good shapes along with good resolution. For this case authors conclude that 1.3ml/min. flow rate is optimum range which is subsequent in less retention time, stability at the baseline and less noise. After the application of this proposed process, retention time for the Impurity – A is noted at 6 min, for Impurity B is noted at 11min and for Impurity – C is noted at 4min. quantitative linearity and range was obeyed in the concentrations of LOQ as 7.407µg/mL to 150% i.e., 44.440µg/mL for Nob. The relevant regression equation value to be given Impurity – A, regression (R) as 0.99998 and Y-Intercept at 100% concentration 3.24; Impurity – B, regression (R) as 0.99998 and Y- Intercept at 100% concentration -0.65; Impurity – C, regression (R) as 1.0000 and Y- Intercept at 100% concentration -1.16. Both the intra-day, inter-day drug variations by this proposed method revealed that an RSD less than Impurity – A 0.9%, Impurity – B 2.1%, and Impurity – C 2.4%, indicating that the method is precise. This method tolerated minor changes in optimized chromatographic circumstances which is indicating that robustness is good. This reveals that the column is efficient. There are no interfering peaks identified in different chromatograms that indicate that the excipients utilized in the tablet formulations won't interfere by estimation of this drug by this projected LC-MS method. This LC - MS method is identified to be more simple, high precise, high accuracy and more sensitive to determine Nob. This method is validated as per rules and regulations given by ICH. Total parameters described in this work met the acceptance criteria. Applicability of this method for simultaneous estimation of Nob in tablet dosage forms is finalized. Hence, this method is specific and should successfully be used to estimate Nob in bulk drug substance, pharma dosage forms. Due to this reason this prospective process should be conveniently adopted to routine quality control analysis of Nob drugs.

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7. CONFLICTS OF INTEREST:

There are no conflicts of interest among the authors who have done this present work.

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