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LC-MS/MS method for determination of potential genotoxic impurities in imatinib mesylate

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ABSTRACT

*The objective of the present work was to develop LC-MS/MS method for the determination ofMethyl-3-N [4-(3-Pyridinyl)-2-pyrimidinyl]-1,3-benzenediamine(**PNMP**) and 4-(4-Methylpiperazinomethyl) benzoic acid dihydrochloride(**MPBA**) content in Imatinib Mesylate on YMC –Basic, 250 X 4.6 mm 5µm column using a gradient mixture of solvent A (10mM ammonium formate , adjust the pH of the above solution to 7.0 using Aq.NH3) and solvent B (Methanol(70): Acetonotrile(30)). The flow rate is 1.0ml/min. Statistical analysis proved the method to repeatable, specific and accurate for estimation of **PNMP** and **MPBA** content. It can be used as a LC-MS/MS method due to effective quantification method for trace level potential genotoxic impurities.*

Keywords: LCMS/MS, Imatinib Mesylate, Methyl-3-N-[4-(3-Pyridinyl)-2-pyrimidinyl]-1,3-benzene diamine (**PNMP**), 4-(4-Methylpiperazinomethyl) benzoic acid dihydrochloride(**MPBA**).

INTRODUCTION

Imatinib Mesylate chemically Known as 4-[(4-methylpiperazin-1-yl) methyl]-N-(4-methyl-3-{[4-(pyridin-3-yl) pyrimidin-2-yl] amino} phenyl) benzamide, has an empirical formula of $C_{29}H_{31}N_7O.CH_3SO_3H$ and a molecular weight of 589.72. Imatinib Mesylate is a tyrosine-kinase inhibitor used in the treatment of multiple cancers, most notably Philadelphia chromosome-positive (Ph+) chronic myelogenous leukemia (CML) [1]. Like all tyrosine-kinase inhibitors, Imatinib Mesylate 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 [2].

Imatinib Mesylate is used in chronic myelogenous leukemia, gastrointestinal stromal tumors (GISTs) and a number of other malignancies [3]. The U.S. Food and Drug Administration (FDA) have approved Imatinib Mesylate as first-line treatment for Philadelphia chromosome (Ph)-positive CML, both in adults and children. The drug is approved in multiple Ph-positive cases CML, including after stem cell transplant, in blast crisis, and newly diagnosed [4]. Imatinib Mesylate was approved for use after the surgical removal of KIT-positive tumors to help prevent recurrence [5]. Imatinib Mesylate may also have a role in the treatment of pulmonary hypertension. It has been shown to reduce both the smooth muscle hypertrophy and hyperplasia of the pulmonary vasculature in a variety of disease processes, including port pulmonary hypertension [6]. Mouse animal studies at Emory University in Atlanta have suggested that Imatinib Mesylate and related drugs may be useful in treating smallpox, should an outbreak ever

occur [7].

In vitro studies identified that a modified version of Imatinib Mesylate can bind to gamma-secretase activating protein (GSAP), which selectively increases the production and accumulation of neurotoxic beta-amyloid plaques[8].

Besides the reported impurities, the starting materials Methyl-3-N[4-(3-Pyridinyl)-2-pyrimidinyl]-1,3-benzenediamine (***PNMP***) and 4-(4-Methylpiperazinomethyl) benzoic acid dihydrochloride (***MPBA***) are also to be monitored in the drug. Both these impurities are potential genotoxic impurities [9], hence the impurity levels are to be monitored at ppm levels. Hence, LC-MS/MS method for determination of ***PNMP*** and ***MPBA*** content was developed and validated as per international conference on harmonization (ICH) guidelines [10].

MATERIALS AND METHODS

All reagents were obtained commercially and were of the highest commercial quality and used without further purification. Solvents were freshly distilled and used. TLC or HPLC routinely checked the purity of all compounds. IR spectra were recorded on a Perkin-Elmer model spectrum100 instrument. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100MHz) spectra were recorded in $CDCl_3$ or DMSO using Brucker instrument and Mass spectra were recorded on a ABSciex mass spectrometer model: API2000 operating at 70 eV.

Chemicals and reagents

Imatinib Mesylate and its impurities viz. ***PNMP*** and ***MPBA*** were obtained from Suven Life sciences Ltd. Ammonium formate, Aq.ammonia, Methanol and Acetonitrile were obtained from Rankem, New Delhi, India. All solutions are prepared in Milli Q water (Millipore USA).

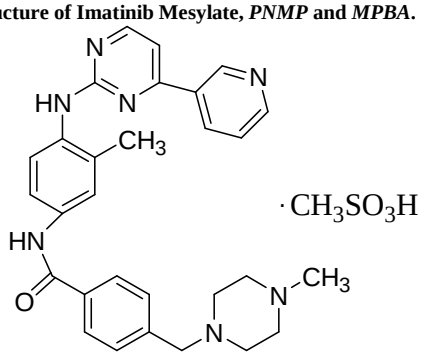
LCMS instrumentation and conditions

Shimadzu SIL-HTC separation module (Shimadzu Corporation) equipped with API 2000-LC-MS/MS (MDS SCIEX) with Analyst software was used for the analysis. YMC-Basic column (250 X 4.6 mm, 5um YMC Corporation, Japan) and a gradient mixture of mobile phase A and B were used as stationary and mobile phases, respectively. Buffer contains 10mM ammonium formate; adjust the pH to 7.0 using Aq.NH_3. Buffer was used as solvent A. Methanol:Acetonitrile(70:30) was used as solvent B. The gradient program (T/%B) was set as 0.01/10, 5/10, 10/50, 15/50, 20/60, 25/60, 27/10 and 40/10. 1.0ml/min flow rate and 20µL of injection volume were maintained. The eluted compounds were monitored at MRM transitions for ***PNMP***: 278.1 $\pm$ 0.5(parent), 106.1 $\pm$ 0.5(Product) and ***MPBA***: 235.1 $\pm$ 0.5(parent), 135.0 $\pm$ 0.5(Product). The column oven temperature was maintained at 30°C.

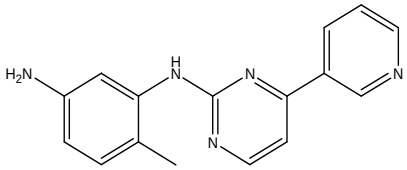
Preparation of standard solutions

Mobile phase (A: B) (70:30) was used as diluent. 0.00008mg/ml solution of ***PNMP*** and ***MPBA*** was prepared in diluent for system suitability. A blend of ***PNMP*** and ***MPBA*** 0.001% was prepared in diluent with respect to 8.0mg/ml of Imatinib Mesylate

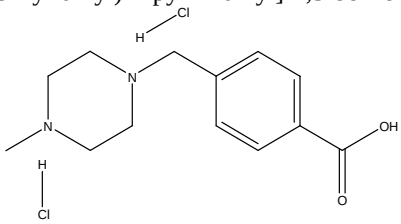
Fig-1: Structure of Imatinib Mesylate, *PNMP* and *MPBA*.



4-[(4-methylpiperazin-1-yl) methyl]-*N*-(4-methyl-3-{[4-(pyridin-3-yl) pyrimidin-2-yl] amino} phenyl) benzamide (Imatinib Mesylate)



4-Methyl-3-N-[4-(3-Pyridinyl)-2-pyrimidinyl]-1,3-benzenediamine (***PNMP***)



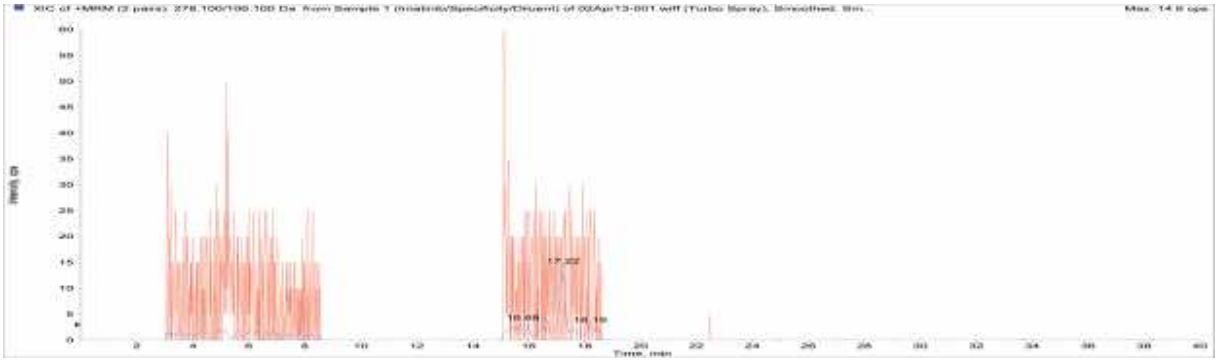
4-(4-Methylpiperazinomethyl) benzoic acid di-hydrochloride (***MPBA***)

LC-MS/MS method development

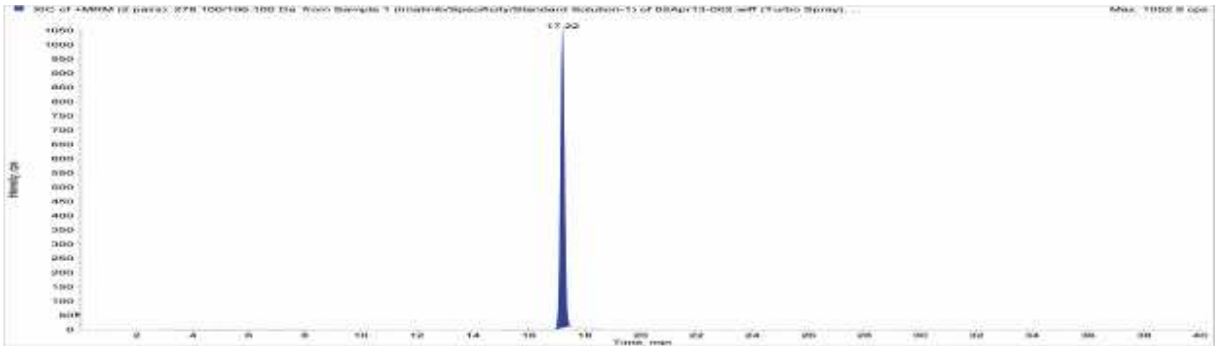
The LC-MS/MS method was optimized so as to obtain a method that can resolve impurities from Imatinib Mesylate. The buffer having pH 7.0 was adopted, because, it was suitable to separate the impurities from Imatinib Mesylate. YMC-Basic (250 X 4.6 mm, 5um) column allowed to a rapid resolution between all the impurities.

Fig-2: A typical chromatogram of Blank, *PNMP* individual, *MPBA* individual, *PNMP* in test sample, *MPBA* in test sample, *PNMP* in spiked test sample and *MPBA* in spiked test sample

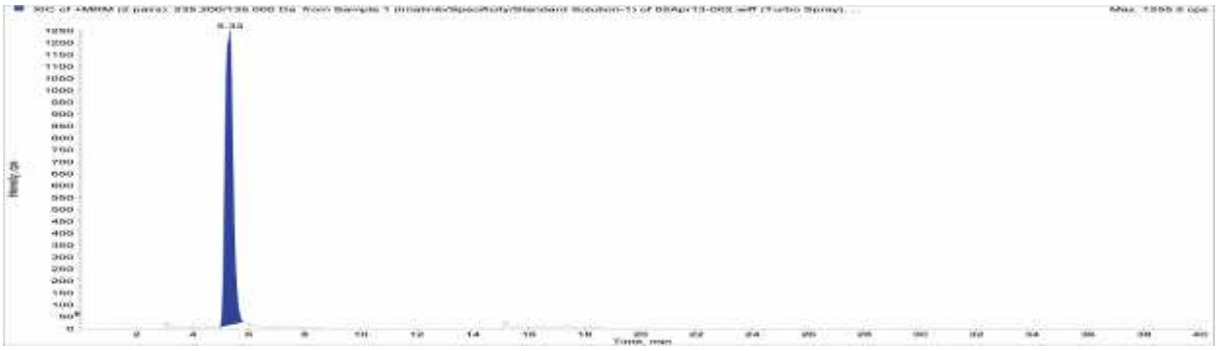
Blank



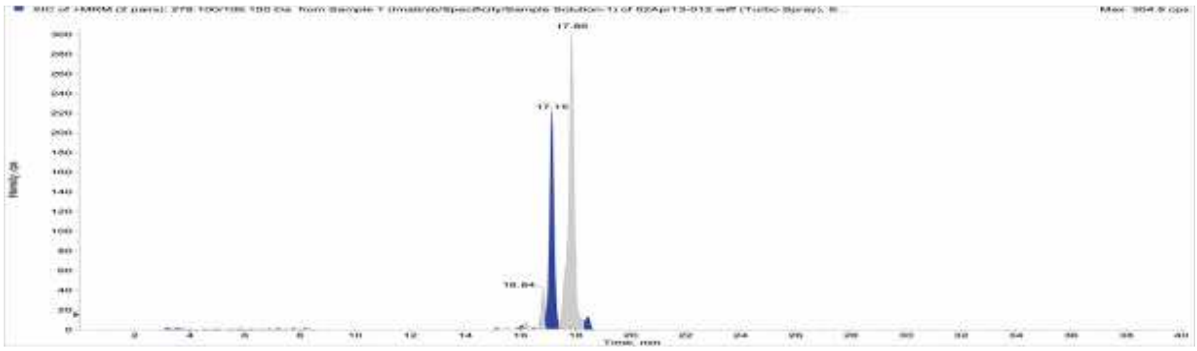
PNMP



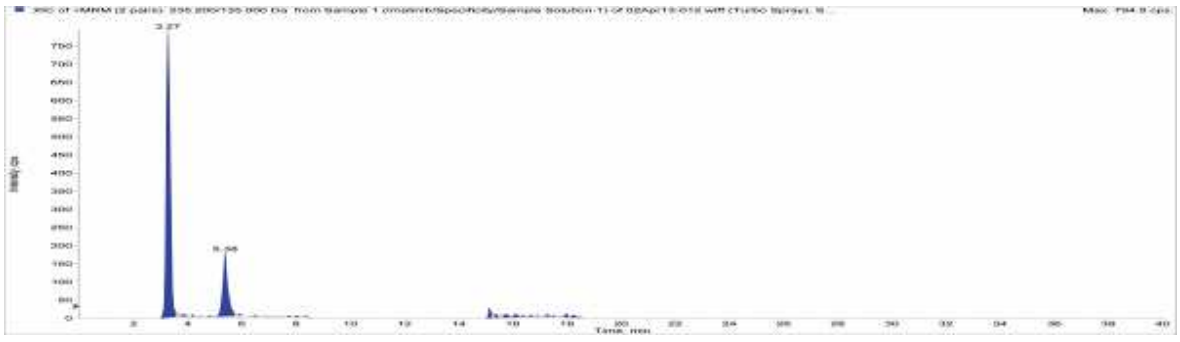
MPBA



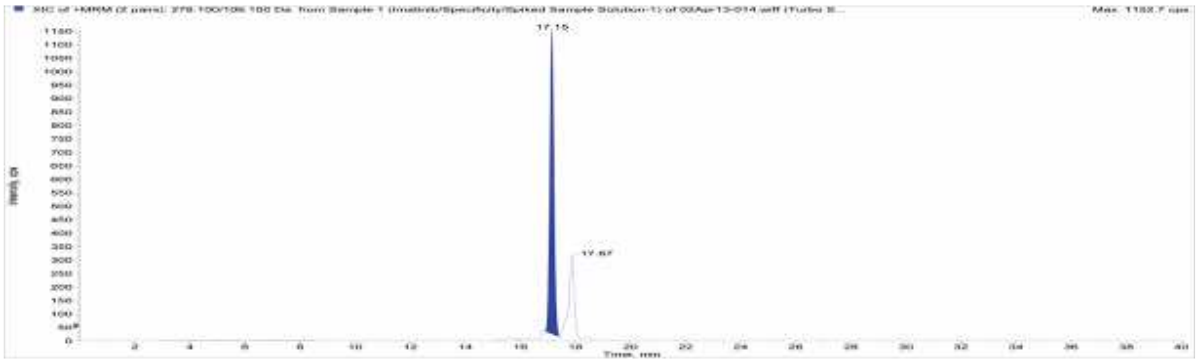
PNMP in Test sample



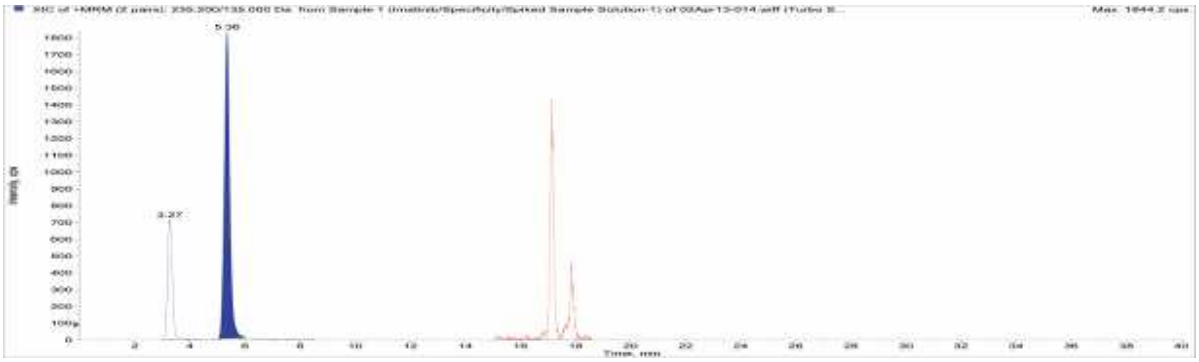
MPBA in Test sample



PNMP in Spiked sample



MPBA in Spiked sample



Method Validation

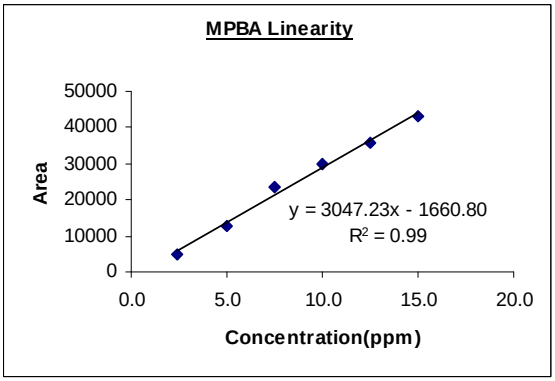
The developed method was validated as per ICH guidelines and the results are given (Table-I). The specificity of the developed LCMS method for Imatinib Mesylate was determined in the presence of its process impurities. All the analysis was carried out by LCMS with API 2000-LCMS/MS. The detection limit (DL) and quantification limit (QL) for ***PNMP*** and ***MPBA*** were determined at a signal to noise ratio of 3:3 and 10:1 respectively, by using Analyst software. Precision study was carried at QL level by injecting six times and calculating the percentage of R.S.D of area of ***PNMP*** and ***MPBA***. Linearity test solutions for six concentration levels from QL to 150 % of the specification level (0.001%). Peak area versus concentration data was performed by least-squares linear regression analysis. Standard addition and recovery experiments were conducted to determine accuracy of impurities quantitation in bulk drug samples. The study was carried out in triplicate at QL, 100% and 150% level with respect to specification 0.001%. The percentages of recoveries for impurities were calculated. The robustness of developed method was determined by altering experimental conditions purposely and evaluating the resolution between Imatinib Mesylate and all impurities. Flow rate was changed by $\pm$ 0.1 units, pH was varied by $\pm$ 0.1 units, and column temperature was studied at 28°C and 32°C instead of 30°C in all above varied conditions the components of the mobile phase were held constant and no significant change (relative error less than 5%) of relative retention time was observed. Significant changes were not observed in all the impurities. The data confirmed that sample solutions were stable up to 24hrs. The system suitability was established in terms of %RSD for ***PNMP*** and ***MPBA*** which was not more than 15.0.

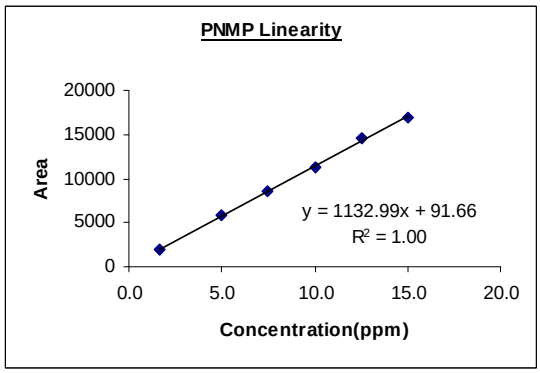
Table-I Validation data of the developed method

Parameter	***PNMP***	***MPBA***
DL(PPM)	0.54	0.78
QL(PPM)	1.64	2.37
Method Precision(%RSD)#	9.0	5.9
Accuracy [a] (%recovery) at:-		
QL (%)	95	104
100 (%)	85	91
150 (%)	87	89

[a] *Carried at QL, 100% and 150% level with respect to specification (0.10% i.e10ppm)*
DL: Detection limit, QL: Quantification limit

Fig-3: Linearity graphs for *MPBA* and *PNMP*





CONCLUSION

LC-MS/MS method has been developed and validated for estimation of PNMP and MPBA in Imatinib Mesylate. This method is able to separate the Imatinib Mesylate from its impurities; it can be conveniently applied for the testing of batch release.

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