Biological Forum – An International Journal **7**(1): 1820-1827(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Determination of Imatinib and its Genotoxic Impurities in Tablets

Fatma Ghrib*,**, Aymen Chtioui*, Taieb Saied** and Nizar Bellakhal**

**Département d'Oncologie, Néapolis Pharma: Les Laboratoires MédiS, Route de Tunis - Km7- BP Nabeul, Tunisie.*
***Unité de Recherche de Catalyse, d'Electrochimie, de Nanomatériaux, et leurs Applications et de Didactique (CENAD), Institut National des Sciences Appliquées et de Technologie (INSAT), Centre Urbain Nord BP Tunis Cedex, Tunisie.*

(Corresponding author: Fatma Ghrib)
(Received 27 April, 2015, Accepted 07 June, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The determination of Imatinib Mesylate and its genotoxic impurities were achieved using a new, simple and sensitive liquid chromatographic method. The analyses were performed using the analytical column Zorbax Eclipse XDB C18 (250 × 4.6 mm, 5 µm). Mobile phase was composed of 30 mM octane sulphonic acid in 10 mM aqueous KH_2PO_4 (pH 2.3) and Acetonitrile. An isocratic mode was adopted for the assay and a gradient one for the degradation. Detection was performed on both Photodiode Array and DUAL UV-Vis detectors at 267 nm for the assay and 234 nm for the degradation study. The two methods were validated in terms of specificity, linearity, accuracy, repeatability and intermediate precision. The limit of detection and quantification were calculated for the degradation essay.

Keywords: Imatinib Mesylate; genotoxic impurities; assay; method validation; anticancer drugs

INTRODUCTION

As novel synthesized drug, there are only few methods in the literature for Imatinib Mesylate quantification in pharmaceutical dosage forms (Medenica *et al.*, 2004, Rosasco *et al.*, 2005, Ivanovic *et al.*, 2004) and for its purity evaluation in bulk drug (Vivekanand *et al.*, 2003). It was approved by US Food and Drug Administration (FDA) in 2001(Habeck, 2002). and it is recently official in European Pharmacopoeia as active substance only in April 2015.

Imatinib Mesylate is a protein-tyrosine kinase inhibitor. It is useful for the treatment of chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs) and some other diseases (Ksienski, 2010). It is designated chemically as 4-[(4-Methyl-1-piperazinyl) methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl] amino]-phenyl] benzamide methanesulfonate with empirical formula $C_{29}H_{31}N_7O.CH_4O_3S$. His chemical structure is given in Fig.1. The usual tablet dose is 100 mg and 400 mg.

This work is made in order to develop and validate a liquid chromatographic method for the simultaneous determination of this anticancer drug in the presence of its degradation products in film-coated tablets. From the literature, these impurities were observed to be a process impurities and genotoxic (Yadav *et al.*, 2012). The method was validated following the analytical performance parameters suggested by International Conference on Harmonisation, 1995, 1996, 2005, Caporal *et al.*, 1992).

MATERIAL AND METHODS

A. Drug and reagents

Samples of Imatinib Mesylate and its two process impurities 4-[(4-Methyl-1-piperazinyl) methyl] benzoic acid dihydrochloride (impurity1 acid) and N-[(2-Methyl-5-(4-((4-methylpiperazin-1-yl) methyl) benamido) phenyl)-4-((4-methylpiperazin-1-yl)methyl)-N-(4-(pyridine-3-yl)pyrimidin-2-yl)benzamide (impurity 2 dimer) were procured from Dr. Reddy's Laboratories Limited. Chemical structures of these related substances are shown in Fig. 2. Methanol (HPLC grade) and acetonitrile (HPLC grade) were purchased from VWR Chemicals (EC). However, the analytical reagent grade potassium dihydrogen ortho phosphate was obtained from Sigma-Aldrich (Germany) and the standard reagent sodium octane sulphonic acid from Carlo Erba, (France). HPLC grade ortho phosphoric acid was purchased from Fluka Analytical (Switzerland). HPLC grade water was obtained from Millipore Milli Q plus purification system.

B. Equipment

The analyses were carried out on Waters Liquid Chromatography System (Alliance e2695 Model) equipped with a photodiode array detector (a2998) which is used for the development trials and a2489 DUAL UV-Vis one for validation studies. The chromatographic data were monitored with the EMPOWER 3 manager software.

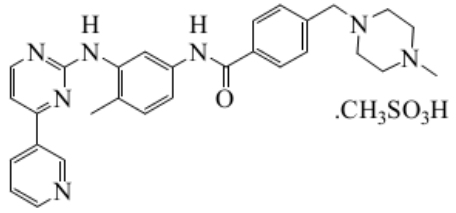
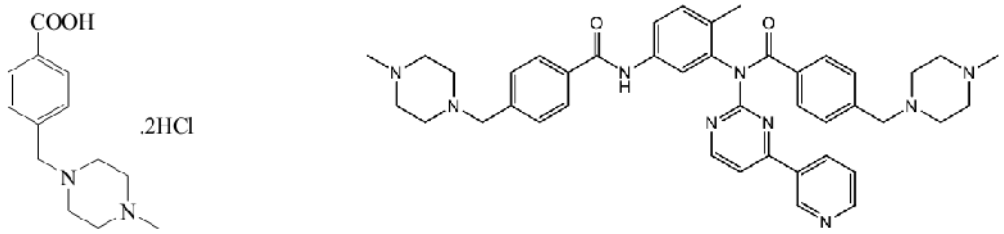


Fig. 1: Chemical structure of Imatinib Mesylate.



Impurity1: Acid Impurity Impurity2: Dimer Impurity

Fig. 2. Chemical structures of Imatinib Mesylate impurities.

To achieve the development and validation studies, we have also used a pH meter (Mettler Toledo; model: SE S470-K), an analytical balance (Mettler Toledo; model: XSE205 dual range) and a sonicator (ISOLAB; model: 621.02.001).

C. Preparation of mobile phase

The mobile phase used for development essays and for validation studies was the same. However for the dosage essay the ratio of the composition was different from the degradation one. It contained aqueous buffer solution and acetonitrile.

Aqueous buffer solution was a mixture of 30 mM octane sulphonic acid and 10 mM aqueous potassium dihydrogen phosphate buffer. The pH of this solution was adjusted to 2.3 with ortho phosphoric acid and then this buffer solution was filtered through a membrane filter with a porosity of 0.45 µm and degassed by sonication.

D. Column Choice

During development studies for assay and degradation of the Imatinib Mesylate to its impurities, we tested two columns. The first one was Symmetry Shield RP18 (150 × 4.6 mm, 5 µm) from Waters Technology as reported in the literature [4]. The second column was Zorbax Eclipse XDB C18 (250 × 4.6 mm, 5 µm) from Agilent Technology which has fulfilled a better result leading to an optimized method.

E. Development Method

Chromatographic conditions and preparation of samples during the assay: As we have mentioned before, we have used the Symmetry Shield column and then the Zorbax with a flow rate of 1.0 mL/min and a temperature maintained to 35°C. The injection volume was set to 15 µL and the detector was set at a wavelength of 267 nm. The composition of mobile phase was at the ratio of 60 % of the aqueous buffer solution and 40 % of acetonitrile. Stock solutions of Imatinib were prepared in methanol in the concentration of 0.1 mg/mL.

Chromatographic conditions and preparation of samples during the degradation of Imatinib Mesylate to its impurities: For both columns, we proceeded such as for the assay with some modifications like the flow rate and the wavelength which were set at 0.8 mL/min and 234 nm, respectively. The gradient program was described bellow in Table 1. Stock solutions of Imatinib and its two impurities (Acid and Dimer) were prepared in methanol with a concentration of 1 mg/mL and 0.002 mg/mL, respectively.

Table 1: Gradient Program.

Time (min)	(%) Aqueous Buffer Solution	(%) Acetonitrile
0.01	65	35
08.0	70	30
25.0	70	30
35.0	50	50
45.0	65	35

RESULTS AND DISCUSSION

A. Method development and column selection for assay and degradation methods

Two different columns were employed, as we have mentioned before, to develop a suitable HPLC method for the determination of Imatinib Mesylate and for its human carcinogen related substances. This essay was tried to get good peak shapes and selectivity for Imatinib Mesylate and his acid and dimer impurities.

The first column Symmetry Shield RP18 (150 × 4.6 mm, 5 µm) has performed a good peak separation; as mentioned in the literature cited before [4]. The obtained retention time (RT) was 7.173 min although those in the literature were around 11 and 13 min. The symmetry factor was equal to 1.21 with 5526 theoretical plates. Peak's area was 4845905 for 0.1 mg/mL of the mentioned substance (Fig. 3).

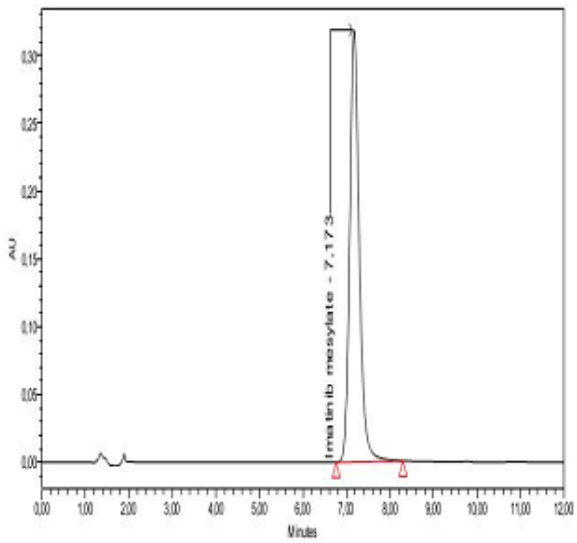


Fig. 3: Typical chromatogram of Imatinib Mesylate in Symmetry Shield RP18 (150 × 4.6 mm, 5µm) column under chromatographic conditions adopting for the dosage essay.

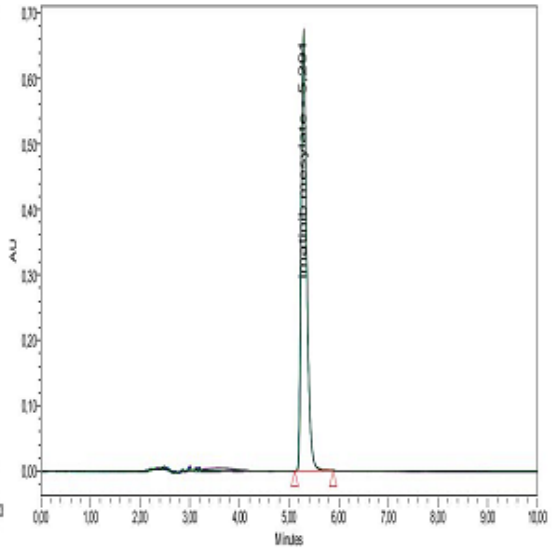


Fig. 4: Typical chromatogram of Imatinib Mesylate in Zorbax Eclipse XDB C18 (250 × 4.6 mm, 5µm) column under chromatographic conditions adopting for the dosage essay.

Results obtained with Zorbax Eclipse XDB C18 (250 × 4.6 mm, 5 µm) show that Imatinib Mesylate eluted around 5.0 min which was optimized comparing to the one separated with Symmetry Shield RP18. Theoretical plates were doubled to 10621 and USP tailing was found to be 1.42. The area of the peak was 5128805 for the same amount of Imatinib Mesylate (Fig. 4).

We can conclude that although Symmetry Shield RP18 has yielded satisfactory results, Zorbax Eclipse XDB C18 realized a better result at the same chromatographic conditions and thus an optimized method for the determination of Imatinib Mesylate and its related substances.

Consequently, for validation study of both assay and degradation of Imatinib Mesylate to its impurities, we will use Zorbax Eclipse XDB C18 (250 × 4.6 mm, 5 µm).

B. Method Validation

The method validation was performed according to International Conference on Harmonisation (ICH) guidelines for validation of analytical procedures. All samples were analyzed using the assay and the degradation chromatographic conditions described previously in this work.

No interference from the sample recipients could be observed at this two detection wavelength.

Specificity: Concerning the assay validation, specificity was evaluated by comparing the chromatograms and the data obtained from the diluent, the placebo solution, the standard solution (analyte: Imatinib Mesylate) and the test solution (Pharmaceutical Reconstituted Form), as showed in Fig. 5.

The comparison showed that there is no interference between the peak due to Imatinib Mesylate (RT ~ 5.0 min) and those due to the diluent and the placebo. Therefore, we can confirm that the method of determination of Imatinib Mesylate in the film-coated tablet by HPLC is specific.

The specificity of the method of the determination of degradation products in Imatinib Mesylate in the film-coated tablet was also studied.

It was achieved by comparing the chromatograms and the data obtained from the diluent, the placebo solution, the solution for peak identification (containing Imatinib Mesylate, impurity1 and impurity2), the standard solution (Fig. 6) and the test solution (Fig.7). The comparison of the chromatograms obtained with the diluent, the placebo solution, the standard solution, the solution for peak identification and the test solution showed the absence of interference between the peak due to Imatinib Mesylate and those due to the diluent, the placebo and impurities (impurity1 and 2). In addition, the resolution factor between the peak due to Imatinib Mesylate (RT = 32 min) and that of the impurity 2 (RRT = 1.09) is equal to 4.6.

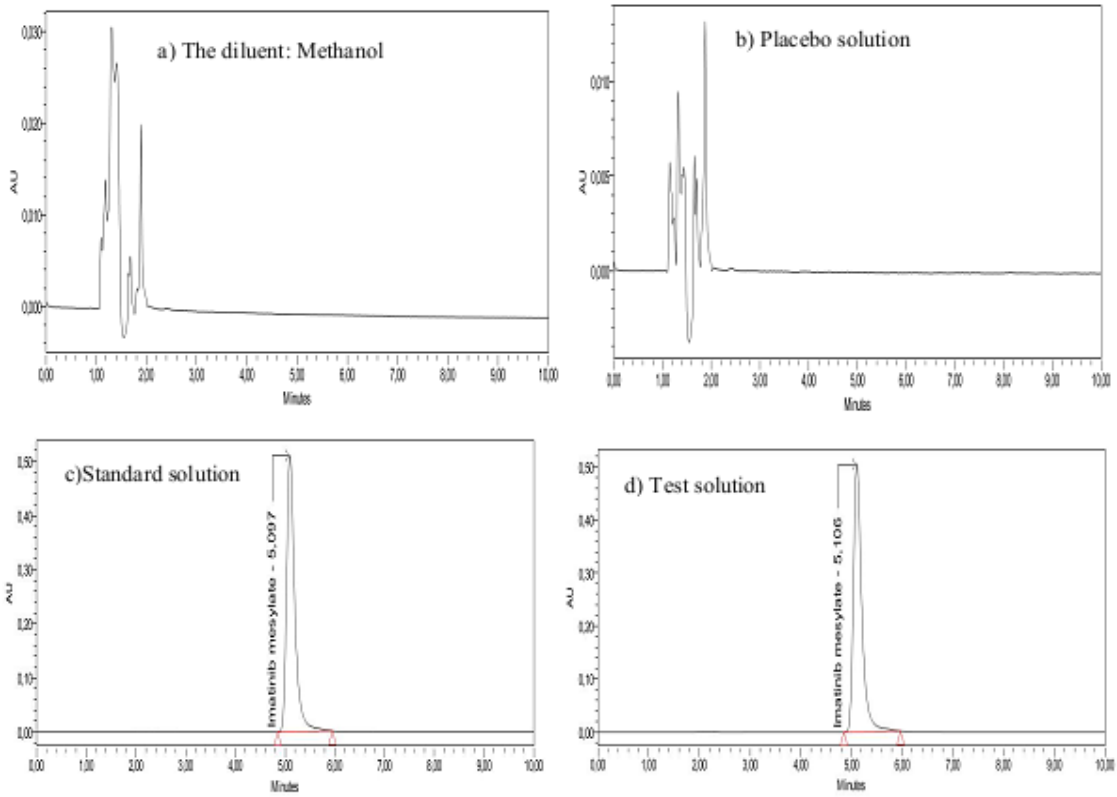


Fig. 5. Typical HPLC chromatograms.
Chromatographic conditions: mobile phase: acetonitrile/buffer (40:60; v/v); stationary phase Zorbax Eclipse XDB C18 (250 × 4.6 mm, 5µm); flow rate: 1 mL/min; T = 35 °C and detection UV: 267 nm

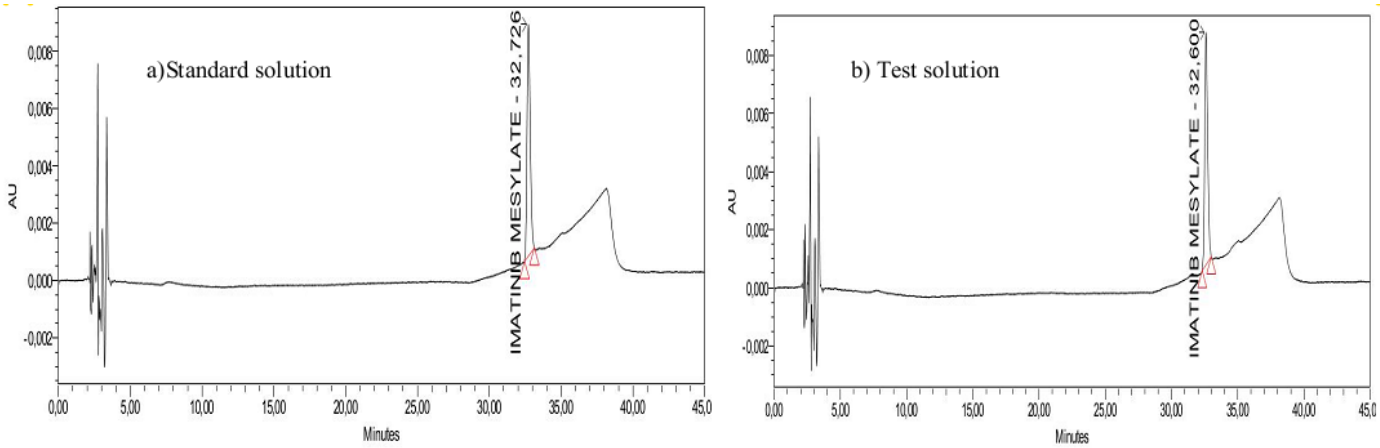


Fig. 6. Typical HPLC chromatograms under degradation chromatographic conditions.

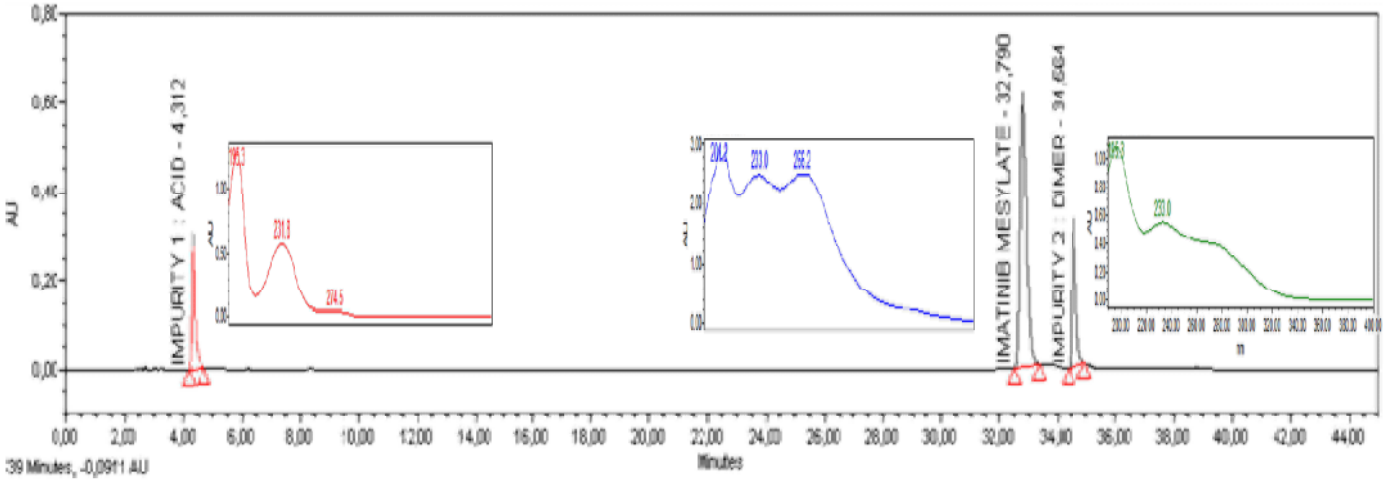


Fig. 7. Typical HPLC chromatogram and UV spectra of the solution containing Imatinib Mesylate, impurity1 and impurity2 under degradation chromatographic conditions.

We can conclude that the method of the determination of degradation products in Imatinib Mesylate in the film-coated tablet is specific.

Linearity: Linearity was investigated by preparing solutions at five spaced concentration levels which cover the interval of validation. This interval was represented by the concentrations of 60 %, 80 %, 100 %, 120 % and 140 % for the validation of the assay and by 0.10 %, 0.20 %, 0.30 %, 0.40 %, and 0.50 % around the content accepted individual impurity limit (0.2 %) for the validation of the degradation method. Three independent series of five concentrations was carried out with a one serie per day. The evaluation of this criterion was studied on the analyte (Imatinib Mesylate) and on the pharmaceutical reconstituted form (Imatinib Mesylate + matrices) for the both methods.

Linearity of the detector responses was determined by preparing calibration graphs and regression equations. The slope, intercept of the straight line and regression equations are summarized in Table 2. The correlation coefficients between the concentration of the drug and detector response are found to be higher than 0.995 (Caporal *et al.*, 1992).

This linearity should be verified by the following statistical tests: Cochran test, Student's t-test and Fisher test. The statistical evaluation of the linearity study is presented in Table 2 as well. The obtained statistical parameters demonstrated that the two methods (the determination of Imatinib Mesylate and of its impurities) have a good linearity over the considered concentration range.

Table 2: Statistical parameters of the linearity of Imatinib Mesylate

	Assay		**Degradation of Imatinib Mesylate to its impurities**	
	Standard Solution	**Test Solution**	**Standard Solution**	**Test Solution**
Slope	203510.3	201298.4	53383	53361
y- Intercept	-167957.7	-102337.6	-3785	-1332.5
Correlation Coefficient	0.997	0.998	0.998	0.999
Regression Equation	203510.3 x -167957.7	201298.4 x -102337.6	53383x-3785	53361x-1332.5
Student's t-test- Comparison of the intercept with 0 *(should be not more than 2.164)*	2.0978 (NS)	1.7903 (NS)	1.6867 (NS)	0.8600 (NS)
Cochran Test - Homogeneity of variances *(should be not more than 0.6838)*	0.5722 (NS)	0.5040 (NS)	0.3461 (NS)	0.4330 (NS)
Fisher Test – Significant slope *(should be not more than 4.67)*	4529 (S)	12118 (S)	8223 (S)	15903 (S)
Fisher Test - Validity of regression line *(should be not more than 3.71)*	1.80 (NS)	0.72 (NS)	2.23 (NS)	0.76 (NS)

NS: Not Significant test, S: Significant test

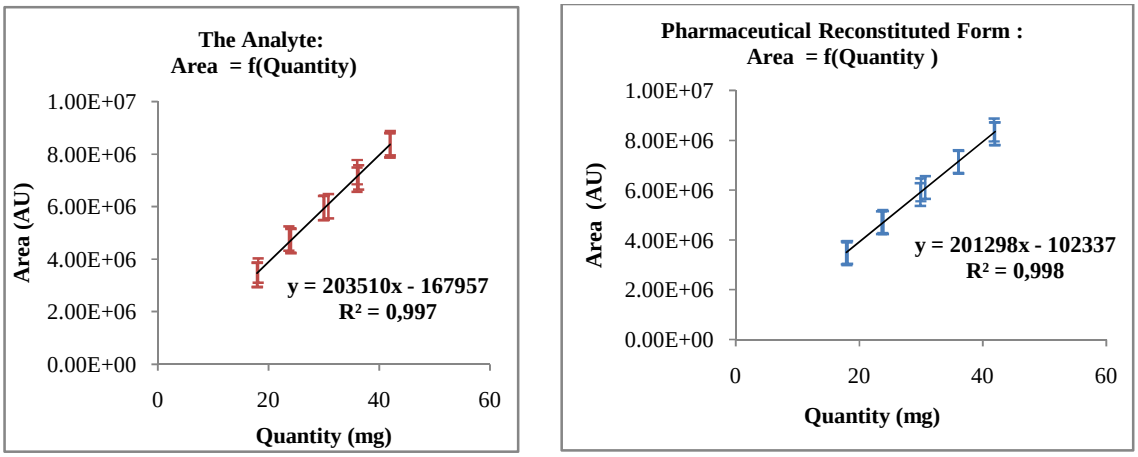


Fig. 8. Calibration Curve of Imatinib Mesylate in standard solution and in pharmaceutical reconstituted form solution (Assay Method).

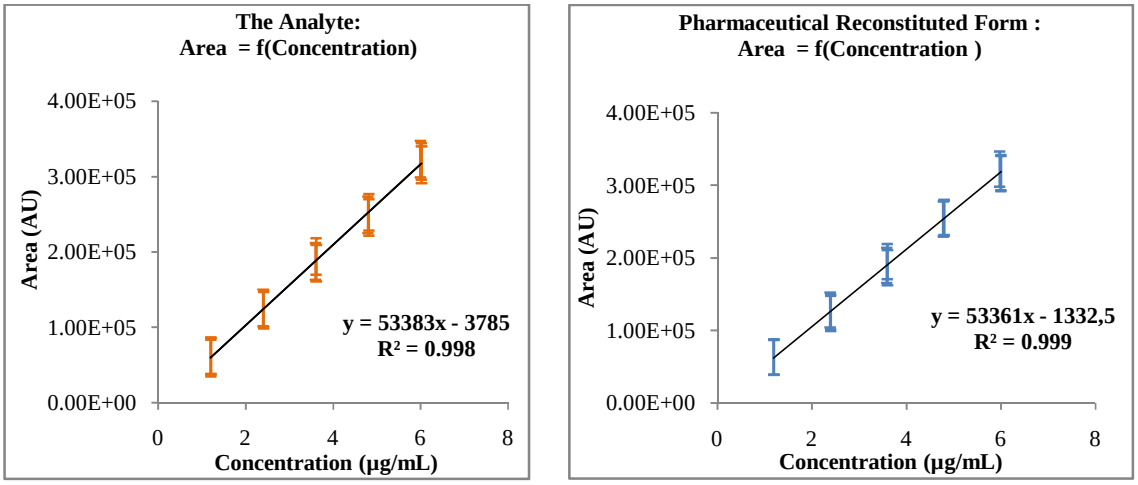


Fig. 9. Calibration Curve of Imatinib Mesylate in standard solution and in pharmaceutical reconstituted form solution (Degradation Method).

The linearity of the peak responses versus concentration was studied from 0.06 to 0.14 mg/mL for both solutions type, for the assay method (Fig. 8). These essays were performed from 1 to 5 µg/mL for the degradation method (Fig. 9).

Accuracy: The accuracy expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. It consists in determining the recovery percentages between the amount (or concentration) found and the amount (or concentration) added, the average recovery and the confidence interval.

The accuracy of the assay and of the degradation were determined by comparing the found amount (concentration) with the added amount (concentration). The results obtained are shown in Tables 3 and 4. The obtained values confirm the accuracy of the two proposed methods.

Table 3: Accuracy results determined during method validation of the dosage.

Nominal Value (%)	Added Amount (mg)	Found Amount (mg)	Recovery (%)	Average Recovery	Confidence Interval Range (p= 0.95)
60	18	17.96	99.8		
80	23.77	24.02	101.01		
100	30.19	30.27	100.24	***100.06***	***[99.52 ; 100.60]***
120	36.06	35.93	99.64		
140	41.9	41.73	99.58		

Table 4: Accuracy results determined during method validation of the degradation of Imatinib Mesylate to its impurities.

Nominal Value (%)	Added Concentration (µg/mL)	Found Concentration (µg/mL)	Recovery (%)	Average Recovery	Confidence Interval Range (p= 0.95)
0.1	1.196	1.203	100.77		
0.2	2.396	2.396	100.16		
0.3	3.593	3.626	101.04	***100.98***	***[99.96; 102.00]***
0.4	4.793	4.856	101.4		
0.5	5.99	6.076	101.5		

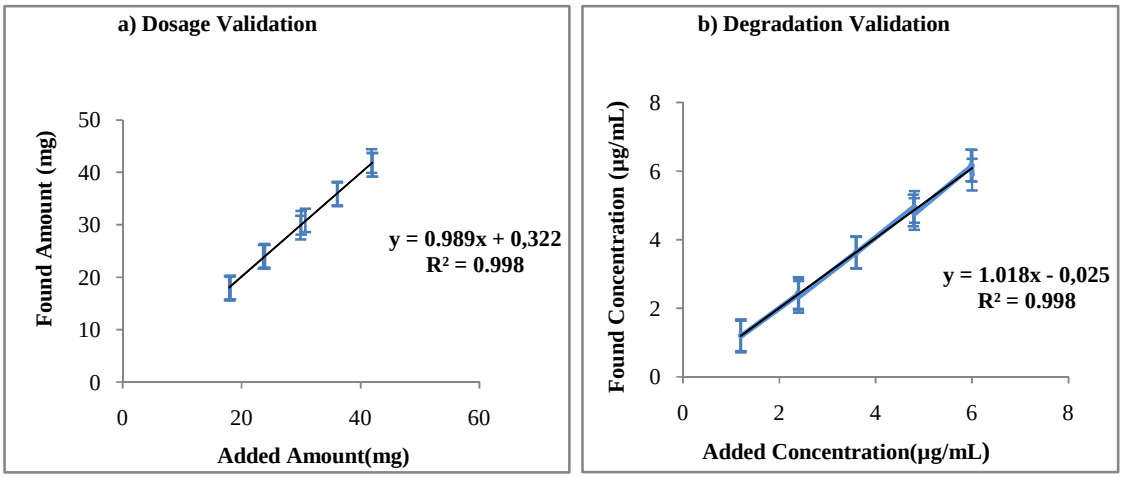


Fig. 10. Concentration of Imatinib Mesylate found against the concentration added.

Method accuracy was also demonstrated by plotting the concentration of Imatinib Mesylate found against the concentration added (Fig. 10).

Precision: Repeatability and Intermediate Precision: To determine the repeatability of the dosage, six representative test solutions with a concentration of 100 % were prepared independently during the same day using the same equipment and with the same operator. The coefficient of repeatability was found to be equal to 1.108. The intermediate precision factor was equal to 1.451. It is based on precision study by changing one or more operating conditions and our parameter to change was the handling day. These calculated values attested the precision of the assay method.

As for the validation of the dosage, the method of determination of Imatinib Mesylate and its impurities in the film-coated tablet by HPLC is precise, with a coefficient of repeatability equal to 1.330 and an intermediate precision factor of 1.573.

Limit of Detection and Limit of Quantification: The LOD and LOQ were determined by using respectively the equations $\frac{3.3\,\sigma}{b}$ and $\frac{10\,\sigma}{b}$, where (σ) is the standard deviation of the response and (b) is the slope of the calibration curve.

Therefore, the LOD was found to be equal to 82.6 ng/mL and the LOQ to be equal to 250.2 ng/mL.

CONCLUSION

Both HPLC methods developed in this study allow the determination of Imatinib Mesylate and its two impurities in the coated tablets. The methods are applicable for qualitative and quantitative Imatinib Mesylate coated tablets. Both have the advantage of being simple, specific, linear, accurate and precise. The obtained results are confirmed by statistical parameters and no interference of the excipients was noted. When compared to previous reported studies in literature, the obtained results appeared to be rapid and highly selective.

ACKNOWLEDGEMENTS

"This project is in the framework of a doctoral thesis MOBIDOC belonging to the PASRI program which is financed by the EU and managed by the ANPR."

The authors gratefully acknowledge the members of the Oncology Department at Medis Laboratories especially Mr. Aymen JELASSI and Mr. Riadh BANI for their encouragement and support to carry out this work.

We wish to thank Miss. Malak MAAMAR and Miss Nouha BEN HAFAIEDH for reviewing and giving suggestions for the manuscript.

REFERENCES

Medenica, M., Jancic, B., Ivanovic, D. & Malenovic, A. (2004). Experimental design in reversed-phase high-performance liquid chromatographic analysis of imatinib mesylate and its impurity. *Journal of Chromatography A*. **1031**, 243-248.

Rosasco, M. A., Moyano, M.A., Pizzorno, M.T. & Segall, A.I. (2005). Validation of an HPLC Method for the Determination of Imatinib Mesylate in Pharmaceutical Dosage. *Journal of Liquid Chromatography & Related Technologies.* **28**: 3283-3292.

Ivanovic, D., Medenica, M., Jancic, B. & Malenovic, A. (2004). Reversed-phase liquid chromatographic analysis of imatinib mesylate and its impurity product in Glivec® capsules. *Journal of Chromatography B.* **800**,253-258.

Vivekanand, V.V., Sreenivas Rao, D., Vaidyanathan, G., Sekhar, N.M., Avijit Kelkar, S. & Ramachandra Puranik, P. (2003). A validated LC method for imatinib mesylate. *Journal of Pharmaceutical and Biomedical Analysis.* **33**, 879 – 889.

Habeck, M. (2002). FDA Licences Imatinib Mesylate for CML. *The Lancet Oncology*. Vol. **3**, no. 1, p.6

European Pharmacopoeia 8.4. , 04/2015:2736

Ksienski, D. (2010). Clinical, Imatinib Mesylate: Past success and future challenges in the treatment of gastrointestinal stromal tumors. *Clinical Medicine Insite Oncology.* **5**: 365-379

Yadav, R.R., Rokade, M.D., Salunke, S.A., Gangrade, D.M., Holkar, G.S. & Daphal, V.N. (2012). Determination of Potential Genotoxic Impurities in Imatinib Mesylate by RP-HPLC Method. *Biological Forum–An International Journal.* **4**(2):15-18.

Guidelines for Industry: Text on Method Validation of Analytical Procedures, ICH Q2A, March 1995.

Guidelines for Industry: Validation of Analytical Procedures: Methodology, ICH Q2B, November 1996.

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Q2 (R1), Validation of Analytical Procedures: Text and Methodology. 2005. Geneva

Caporal Gautier, J., Nivet, J. M., Algranti, P., Guilloteau, M., Histte, M., Lallier, M., N'guyen –Huu, J. J., & Russotto, R. (1992). Guide to analytical validation. Report of an SFSTP commission. II. Examples of application. *STP pharma pratiques.* **2**(4): 227–239.