

ASSESSING IMATINIB MESYLATE IN TWO DIFFERENT SOLID DOSAGE FORMS

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INTRODUCTION

Imatinib mesylate (IM), is a tyrosine kinase inhibitor (TKI), which is used to treat chronic myeloid leukaemia (CML) and other malignant diseases related to over expression of tyrosine kinases protein (Savage and Antman, 2002). Imatinib is one of the first molecular therapies for clinical use that has shown impressive efficacy for CML, advanced gastrointestinal stromal tumours (GISTs), and myeloproliferative disorders associated with PDGFR.

It is approved as a first-line therapy for Ph+ ALL (Iqbal and Iqbal, 2014).

Several RP-HPLC methods can be found in the literature (Rosasco et al., 2005; Rele and Patil, 2019) for its determination in various pharmaceutical forms. The purpose of this work was to evaluate imatinib mesylate in two different solid dosage forms with a new RP-HPLC method developed in our laboratory.

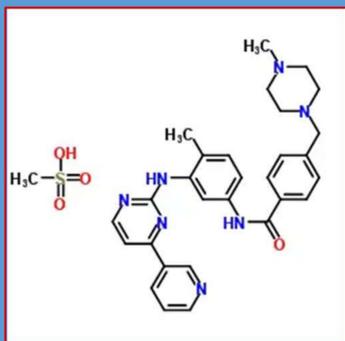


Figure 1: Molecular structure of IM

MATERIALS AND METHODS

Chemicals and equipment

Working standard: Imatinib mesylate (gift from AKBPM). Reagents: metanol (MeOH) (Carlo Erba Reagents), acetonitril (ACN) HPLC grade (Sigma-Aldrich), orthophosphoric acid 85%, distilled water, samples from two different imatinib solid dosage forms.

Chromatographic data were obtained using an Agilent 1200 reversed phase chromatographic system consisting of a High Pressure Mixing Binary Pump, DAD detector and equipped with a ChemStation PC-controlled. The pH measurements were made with a pH meter (Denver Instruments). Centrifuges, mixers and ultrasonic baths were also used. Nylon membrane filters were used for mobile phase filtration (type: Hydrophilic Nylon membrane filter) (EMD Millipore) (47 mm; 0.45 µm pore).

Preparation of standard solutions

100 mg of standard Imatinib was dissolved in methanol in a 100 ml volume flask and was made up to the volume mark with methanol. The obtained solution, at a concentration of 1 mg / ml, was filtered through 0.45-µm porous filters, and stored in suitable containers, refrigerated at 4-8°C. Solutions with concentrations of 5, 10, 20, 30 and 40 µg / ml, were prepared daily for the calibration curves by diluting the stock solution with distilled water. All the solutions obtained were filtered through 0.45 µm pore filters, prior to injection.

Development and optimization of analytic conditions

Many tests were performed to attain optimum conditions. Different column types: LiChrospher® 100 RP-18 (5 µm) LiChroCART® 125-4, ODS HYPERSIL C18 (250 × 4.6) (5 µm), LiChrospher® 60 RP-select B C8 (5 µm) LiChroCART® 250 were tested. Different compositions of the mobile phase components, different pH, as well as changes in column temperature.

Chromatographic conditions

The flow rate was adjusted at 1.0 ml/min and the detection was carried out at 276 nm. The volume of injection was 20 µl.

Method validation parameters

The method was validated according to the ICH validation parameters and was assured to meet the conditions of linearity, accuracy and precision.

Preparation of the samples for the uniformity content

Each tablet is poured into a pre-filled Erlenmeyer flask with 100 ml of distilled water and placed in an ultrasound bath to disintegrate for 45 minutes. A 3 ml sample from each container is transferred in a test tube, which is centrifuged for 5 minutes. An aliquot of 2 ml from the supernatant liquid is further diluted in a 1 to 5 ratio with distilled water until the theoretical concentration reaches 20 µg / ml. The same procedure was applied for 10 tablets of each of the pharmaceutical products, which contain 100 mg of Imatinib. Samples were injected into the apparatus and the data obtained were plotted in graphs to compare the different products.

RESULTS AND DISCUSSIONS

Literature review on the characteristics of Imatinib, revealed various evaluations with HPLC (Rosasco et al., 2005; Rele and Patil, 2019). Unfortunately, none of the published methods could be carried out in our laboratory, hence to evaluate imatinib in solid dosage forms a new method was developed.

The tests revealed that the LiChroCART® 250-4 LiChrospher® 60 RP-select B C8 (5 µm) column resulted the most suitable one. The chosen mobile phase consisted of Acetonitrile / H₂O adjusted to pH 2.2 with orthophosphoric acid in the ratio (32.5: 65.5 v / v).

Tests were carried out at room temperature. The analysis time was 6.5 minutes with imatinib eluting at 4.8 minutes. The method proved sensitive to the pH changes of the mobile phase.

The data obtained showed a consistent and practical method. DAD was used to confirm peak purity, and the result showed a peak with no interference with excipients, degradation products, or impurities.

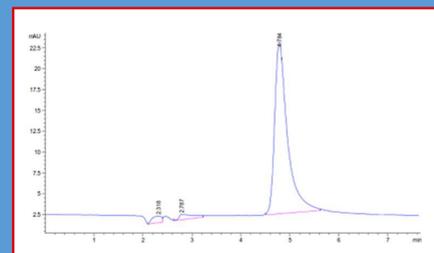


Figure 2: A typical chromatogram of an aqueous solution containing standard Imatinib (5mcg/ml)

Method validation

The linearity of the method was estimated through the correlation coefficient of the linear regression line, and regression equation was $y=68.83x+17.18$ with $r^2 = 0.999$. The method proved specific and selective in assessing Imatinib. Repeated injections ($n = 5$) of each standard solution of 10 µg / ml, 20 µg / ml and 30 µg / ml were performed daily to determine accuracy and precision within day and $n = 18$ between days. All values of RSD for within day and between day tests were below 2.5%.

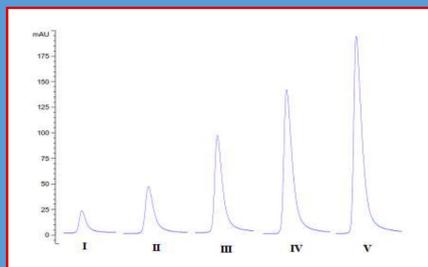


Figure 3: Graphical representation of the chromatograms obtained from standard solutions while testing linearity. Calibration standards containing Imatinib at the following concentrations: I. 5 µg/mL, II. 10 µg/mL, III. 20 µg/mL, IV. 30 µg/mL, V. 40 µg/mL.

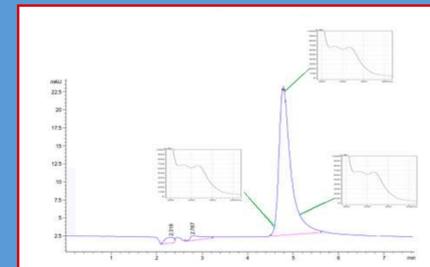


Figure 4: Peak purity assessment using the DAD detector demonstrated in one of the chromatograms of Imatinib.

Table 1: Intraday and interday precision and accuracy.

Nom.Value	Intraday			Interday		
	Measured Value Mean ±SD	Accuracy (Bias)	Precision (RSD %)	Measured Value Mean ±SD	Accuracy (Bias)	Precision (RSD %)
10	10,023 ± 0,225	-0.23%	2,24%	10,095 ± 0,154	-1.0%	1,5%
20	20,25 ± 0,164	-1.23%	0,81%	20,184 ± 0,074	-0.9%	0,4%
30	30,126 ± 0,457	-0.42%	1,52%	29,961 ± 0,238	0.1%	0,8%

Uniformity content of Imatinib tablets

10 tablets from each pharmaceutical product were analysed as defined in European Pharmacopeia 10. The method demonstrates that all the tablets tested are within the specified range respectively between 85% and 115% of the average content.

Table 2: Presenting the upper and lower limits of the measured concentrations and the amounts in mg of Imatinib in the tablets of both products

	Product 1		Product 2	
	Measured conc.	Amount in mg	Measured conc.	Amount in mg
Lower Limit	21.11	84.1	20.96	87.7
Upper Limit	27.20	113.8	28.36	118.6

CONCLUSION

The new method used to evaluate imatinib mesylate was accurate and precise.

The results of the uniformity content for the two pharmaceutical products were within the specified range according to ICH and Ph. Eur.

Determination of Imatinib in pharmaceutical dosage forms with this method enables the opportunity for further studies regarding the dissolution test.

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