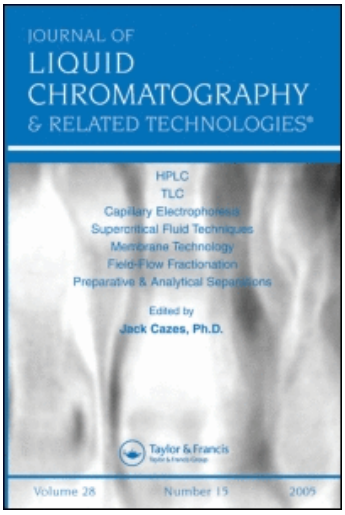

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Validation of an HPLC Method for the Determination of Imatinib Mesylate in Pharmaceutical Dosage

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Abstract: This paper describes the development and validation of a new, simple, fast, and sensitive liquid chromatographic method for the determination of imatinib mesylate. Imatinib mesylate is not listed in any pharmacopoeia, and there are few methods in the literature for its quantitation in pharmaceutical dosage forms. In this work, a C_{18} μBondapak® (3.9 × 150 mm, 5 μm) column was used as the stationary phase, and 30 mM sodium heptane sulphonic acid in 0.01 M KH_2PO_4 (pH 2.5): MeOH (42:58) was the mobile phase. Detection was performed on a UV detector at 237 nm. Through the evaluation of the analytical parameters, it was shown that the method is linear ($r = 0.9994$) at concentrations ranging from 0.3 mg/mL to 0.8 mg/mL. The relative standard deviation values [RSD] for intra- and inter-day precision studies were 1.7 and 2.6. Recoveries ranged between 96.2 and 101.4.

Keywords: Imatinib mesylate, HPLC, Assay, Stability-indicating method

INTRODUCTION

Imatinib mesylate is a protein-tyrosine kinase created by the Philadelphia chromosome abnormality in chronic myeloid leukemia. It inhibits proliferation and induces apoptosis in Ber-Abl positive cell lines, as well as fresh leukemic cells from Philadelphia chromosome positive chronic myeloid leukemias. The chemical name of imatinib mesylate is benzamide,

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4-[(-methyl-1-1piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino] phenyl]-, monomethanesulfonate. The usual tablet dose is 100 mg and 400 mg and the usual capsules dose is 100 mg as Imatinib free base.

As a very novel and recently synthesized drug, there are only a few references for Imatinib mesylate. It was approved by US Food and Drug Administration (FDA) in 1999 and it is not official in any Pharmacopoeia. Most of the analytical techniques for Imatinib mesylate described in the literature are based on the liquid chromatographic determination of this drug in monkey and human plasma,[1–3] in bulk drug,[4] and pharmaceutical formulations.[5,6]

The aim of our investigation was to develop and validate a liquid chromatographic method for the simultaneous determination of Imatinib mesylate in the presence of its degradation products in pharmaceutical dosage forms. The proposed method can be used in a stability assay. The method was validated following the analytical performance parameters suggested by International Conference on Harmonization (ICH).[7]

EXPERIMENTAL

Chemicals and Reagents

Methanol used was HPLC grade (Sintorgan S.A., Argentine). Distilled water was passed through a 0.45 micron membrane filter. Potassium phosphate, monobasic was AR grade (Anedra, Argentine). Sodium heptane sulphonic acid was J. T. Baker (Phillipsburg, NJ).

Solutions and mobile phase were prepared just before use, and all solvents and solutions for HPLC analyses were filtered through a Micron Separations N04SP04700 nylon membrane filter (pore size 0.45 μm) and vacuum degassed before use.

Imatinib mesylate working standard was from Natco Pharma Limited, Chemical Division (Hyderabad, India)

A commercial local capsules formulation was used in this study. Its composition was: Imatinib mesylate 100 mg in a matrix of: colloidal silicon dioxide, crospovidon microcrystalline cellulose, and magnesium stearate.

Chromatographic Conditions and Instrumentation

The HPLC system consisted of a dual piston reciprocating Spectra Physics pump (model ISO Chrom. LC pump), a UV-Vis Hewlett Packard detector (Model 1050), a Hewlett Packard integrator (Series 3395), and a Rheodyne injector (Model 7125). The analytical column was a C_{18} μBondapak® (3.9 × 150 mm, 5 μm). The mobile phase consists of 30 mM sodium heptane sulphonic acid in 0.01 M KH_2PO_4 (pH 2.5):MeOH (42:58). The flow rate was 0.8 mL/min. Detection was performed on a UV detector at

237 nm. The liquid chromatographic method was operated at ambient temperature. The injection volume was 20 μL. In these conditions IM retention time (t_R) was roughly 5.5 min.

Preparation of Solutions

Solutions were prepared on a weight basis and volumetric flasks used as suitable containers in order to minimize solvent evaporation.

Standard Preparation

Standard stock solution of Imatinib mesylate was prepared at a concentration of 0.5 mg/mL, dissolving the appropriated amount of standard in mobile phase.

Sample Preparation

Twenty capsules were weighed and its contents finely powered. An accurately weighed powder sample equivalent to one capsule (100 mg each) was transferred to a 200 mL volumetric flask, 50 mL of mobile phase was added and the flask was kept in an ultrasonic bath for 5 min. The mixture was then diluted to 200 mL with mobile phase, thoroughly mixed, and filtered through a 0.2 μm nylon membrane (25 mm disposable filter; μicroclar, Argentine, Cat. N° Y02025WPH).

Method Validation

System Suitability Test

Relative standard deviations (RSDs) of the area, tailing factor, and retention time were the chromatographic parameters selected for the system suitability test.

Specificity

Forced degradation studies were performed to evaluate the specificity of the method. Degraded samples were prepared by refluxing 2.5 mg/mL of imatinib mesylate working standard with acid (1 N hydrochloric acid), base (1 N NaOH), water, hydrogen peroxide 50 vol, and refluxing for at least 30 min. Both drugs were subjected to thermal (either in solid state and solution in an open container in an oven at 110°C, 48 h) and photochemical degradation (a solution was transferred into a container and exposed to daylight for 48 h). After degradation treatment, samples were allowed to cool at room temperature

and diluted, if necessary, to the same concentration as the standard solution, after being neutralized. After degradation, samples were analyzed using the methodology described in the chromatographic conditions.

Linearity

A stock solution of 2.5 mg/mL of Imatinib mesylate was prepared in a 100 mL volumetric flask by dissolving 250 mg in mobile phase. Appropriate amounts of the stock solutions were diluted with mobile phase, yielding concentrations of 0.30, 0.40, 0.50, 0.60, 0.80 mg/mL. Triplicate injections of each were made in the chromatograph.

Precision

Repeatability was calculated by analyzing 6 samples of the 100% sample preparation. Intermediate precision was assessed by comparing the results obtained by analyzing 6 samples prepared by two different analysts on two different days.

Accuracy

The recovery method was studied at concentration levels of 80%, 100%, and 120% (three samples each). Twenty capsules from the same lot of a commercial formulation were weighed and its contents finely powered. The amount of Imatinib mesylate recovered in relation with the results obtained in the intermediate precision study were calculated.

Robustness

Robustness was established by changing the pH and the mobile phase proportion.

RESULTS AND DISCUSSION

System Suitability

The analytical column was equilibrated with the eluting solvent system used. After an acceptable stable baseline was achieved, the standards and then the samples were analyzed. System suitability results were calculated according to the USP 27 <621> from typical chromatograms.[8] Instrument precision, as determined by six successive injections of the standard preparation, provided a relative standard deviation (RSD) below 1.5%. Peak asymmetry or tailing factor, T, was calculated as $T = W_{0.05}/2f$; where $W_{0.05}$ in the distance from the leading edge to the tailing edge of the peak, measured at

5% of the peak height from the baseline and f is the distance from the peak maximum to the leading edge of the peak. The tailing factor did not exceed 1.5.

Validation Study

Validation of the method was performed according to ICH. This method uses a simple mobile phase. No interference from the sample excipients could be observed at this detection wavelength. All samples were analyzed using the assay chromatographic conditions described.

Specificity

No evidence on interactive degradation products was seen during evaluation. However, Imatinib mesylate showed degradation products after the degradation treatments (Figure 1). Degradation was indicated in the stressed sample by a decrease of the expected value of the drug and increased levels of degradation products. The results of the stress study are presented in Table 1. Selectivity was demonstrated showing that Imatinib mesylate was free of interference from degradation products, indicating that the proposed method can be used in a stability assay (Figure 2).

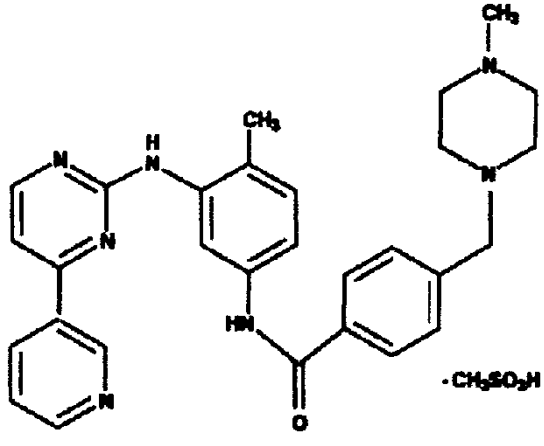

Figure 1. Imatinib mesylate is a protein-tyrosine kinase created by the Philadelphia chromosome abnormality in chronic myeloid leukemia. It inhibits proliferation and induces apoptosis in Ber-Abl positive cell lines as well as fresh leukemic cells from Philadelphia chromosome positive chronic myeloid leukemias Chemical name of Imatinib mesylate is benzamide, 4-[(-methyl-1-1piperazinyl)methyl]-N-[4-methyl-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl]-, monomethanesulfonate (Figure 1). The usual table dose is 100 mg and 400 mg and the usual capsules dose is 100 mg as Imatinib free base.

Table 1. Selectivity: degradation conditions of imatinib mesylate

Condition	Time (h)	Recovery, %	RRT[a] of degradation products
Acid (1 N HCl, reflux)	0.5	89.9	0.332; 0.406; 0.787
Base (1 N NaOH, reflux)	0.5	96.7	0.460; 0.553; 0.726; 3.119
Hydrogen peroxide 50 vol (reflux)	0.5	96.9	0.318; 0.777; 4.094; 4.578
Water (reflux)	0.5	95.0	0.378; 0.802
Dry heat, 110°C	48	96.5	0.382; 0.796
Daylight exposure	48	96.2	0.369; 0.797

[a]RRT, relative retention time.

Range

Assay method range was set at 80 to 120% of the finished product label claim.

Linearity

Linearity of the detector responses was determined by preparing calibration graphs. The linearity of the peak responses versus concentration was studied from 0.3 to 0.8 mg/mL. The representative linear equation was 14139060x – 2855974 with a correlation coefficient (r) of 0.9994, while intercept was not significantly different from zero ($p = 0.05$) (Table 2) (Figure 3).

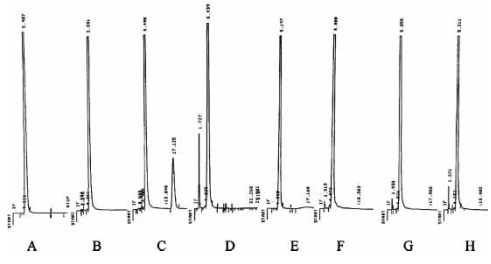

Figure 2. Chromatograms of A) standard, B) acid hydrolysis, C) alkaline hydrolysis, D) oxidation, E) hydrolysis, F) heat (solution) G) heat (solid), H) daylight.

Table 2. Linearity data

Nominal value, %	Injected (μg)	Average peak area response	RSD
60	5.988	82782250.7	0.23
80	7.984	111211360	0.17
100	9.980	136662853	0.25
120	11.980	164115040	0.29
160	15.968	224765840	0.56
Slope[a]	$1.4139 \times 10^7 \pm 8.9890 \times 10^5$		2.0
Intercept[b]	$-2855974.65 \pm 9827999.25$		

[a]Confidence limits of the slope ($p = 0.05$).
[b]Confidence limits of the intercept ($p = 0.05$).

Precision

The precision and accuracy of the assay were demonstrated. The precision is usually expressed as the RSD of a series of measurements. In the study of the instrumental system precision a RSD of 1.4% was obtained. The inter-day precision of the assay was performed by analyzing 6 samples and showed a RSD of 1.7%. In all these cases the RSD obtained was below 1.5%, the limit percentage set for the precision study of the instrumental system, thus showing that the equipment used for the study worked correctly for the developed method, and being highly repetitive.

The intra-day precision was performed by assaying the samples on two different days, by two different analysts. The results were given both

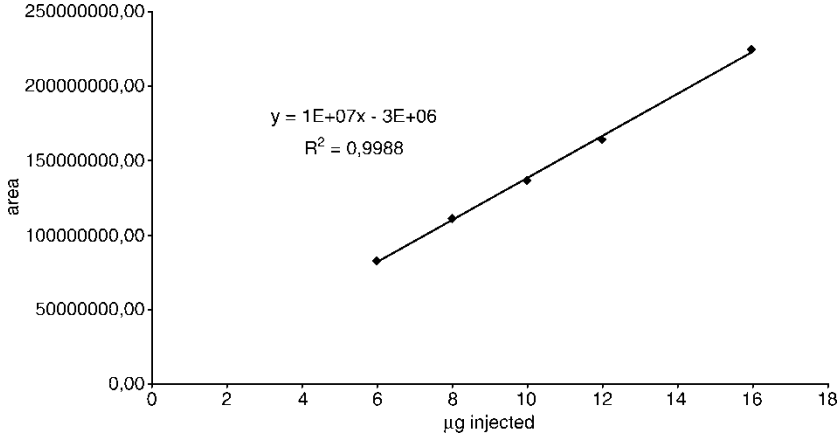

Figure 3. Linearity of assay method.

Table 3. Precision of the assay method

	Analyst 1		Analyst 2	
Sample no.	mg per capsule	RSD (%)	mg per capsule	RSD (%)
1	104.7	0.1	97.8	0.3
2	101.8	0.1	100.7	1.0
3	100.4	0.1	94.1	0.7
4	100.7	0.2	98.7	0.1
5	100.8	0.2	101.4	0.1
6	100.0	0.3	99.3	0.8
Mean	101.4	1.7	98.7	2.6

individually and as the average. For each precision assay, the results were as follows: mean values 101.4 and 98.7 mg per capsule, RSD 1.7% and 2.6%. Test "*t*"; comparing two sample with 95% confidence for 10 degrees of freedom disclosed that both results were not significantly different *inter se* ($t_{n-2,\ \alpha:0.05}$) = 2.23 (Table 3).

Accuracy

The results obtained for the accuracy study (recovery test), with 9 samples of one commercial formulation studied (n = 3 for 80%, 100%, and 120%), indicated that the mean recovery was 98.70% and RSD was 1.7. The

Table 4. Recovery analysis

Nominal value, %	Added amount (mg)	Found amount (mg)	Recovery (%)	Average recovery (n = 3)	RSD (%)
80	44.3	42.6	96.2	98.8	2.6
	40.7	41.3	101.4		
	38.0	37.5	98.8		
100	53.5	52.5	98.1	98.3	1.5
	55.0	54.9	99.8		
	55.4	53.7	96.9		
120	60.5	58.9	97.4	99.1	1.7
	61.5	61.9	100.7		
	57.3	56.8	99.1		
Mean (n = 9)				98.7	1.7

Table 5. Robustness

Mobile phase	RT imatinib (min)
Buffer:methanol 32:68, pH: 2.5	3.3
Buffer:methanol 52:48, pH: 2.5	32
Buffer:methanol 42:58, pH: 3.5	5.8

experimental *t* of the recovery percentage of which the value was 2.25, being below the 2.306 established in the tabulated *t* (95% level of probability, 8 d.f), was also studied. Method accuracy was also demonstrated by plotting the amount (expressed in mg) of imatinib mesylate found against the amount present in capsules of 100 mg. Linear regression analysis rendered slopes not significantly different from 1 (t test, $p = 0.05$), intercepts not significantly different from zero (t test, $p = 0.05$), and $r = 0.9948$ (Table 4).

Robustness

The effect of mobile phase proportion on retention time could be seen in Table 5. An increase of pH did not affect retention time, although the method is very sensible to solvent proportion variation. An increase of the buffer content resulted in longer retention time.

CONCLUSIONS

The method developed in this study has the advantage of being simple, precise, accurate, and convenient. The method employs simple reagents, with minimal sample preparation procedures. The method is applicable for qualitative and quantitative imatinib mesylate capsules. The results obtained are in good agreement with the declared contents. The results are accurate and precise, and confirmed by statistical parameters. There was no interference of the excipients in capsules. The proposed liquid chromatographic method permits simultaneous determination of imatinib mesylate and its degradation products due to good separation and resolution of the chromatographic peaks.

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