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Short communication

Stability-indicating HPTLC determination of imatinib mesylate in bulk drug and pharmaceutical dosage form

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Abstract

A simple, selective, precise and stability-indicating high-performance thin-layer chromatographic method of analysis of imatinib mesylate both as a bulk drug and in formulations was developed and validated. The method employed HPTLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of chloroform:methanol (6:4, v/v). The system was found to give compact spot for imatinib mesylate (R_f value of 0.53 ± 0.02). Densitometric analysis of imatinib mesylate was carried out in the absorbance mode at 276 nm. The linear regression analysis data for the calibration plots showed good linear relationship with $r^2 = 0.9966 \pm 0.0013$ with respect to peak area in the concentration range 100–1000 ng per spot. The mean value ± S.D. of slope and intercept were 164.85 ± 0.72 and 1168.3 ± 8.26 with respect to peak area. The method was validated for precision, recovery and robustness. The limits of detection and quantitation were 10 and 30 ng per spot, respectively. Imatinib mesylate was subjected to acid and alkali hydrolysis, oxidation and thermal degradation. The drug undergoes degradation under acidic, basic, oxidation and heat conditions. This indicates that the drug is susceptible to acid, base hydrolysis, oxidation and heat. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of said drug. The proposed developed HPTLC method can be applied for identification and quantitative determination of imatinib mesylate in bulk drug and dosage forms.

Keywords: Imatinib mesylate; HPTLC; Validation; Stability-indicating; Degradation

1. Introduction

Imatinib mesylate, designated chemically as 4-[(4-methyl-1-piperazinyl)methyl]-*N*-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide methanesulfonate, is a white to off-white to brownish or yellowish tinged crystalline powder [1]. It is a protein kinase inhibitor (PTK) which potently inhibits the Abelson tyrosine kinase. PTK are enzymes that can transfer the terminal phosphate of an adenosine triphosphate molecule to a tyrosine residue of cytoplasmic protein substrate. PTKs are key modulators of cellular signal transduction pathways. If for any reason these signaling proteins are subjected to oncogenic mutation(s), a cellular deregulation may occur, yielding an imbalance between cell proliferation, cell growth and cell death (apoptosis). Hence PTKs have emerged as important therapeutic targets for intervention in cancer [2]. Various methods are available for the analysis of imatinib mesylate in literature like HPLC [3,4], LC–MS–MS [5,6]. But there is no analytical method for estimation of imatinib mesylate in bulk drug and dosage form by HPTLC. Moreover, none of them is stability-indicating method. The International Conference on Harmonization (ICH) guideline entitled 'stability testing of new drug substances and products' requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance [7]. Susceptibility to oxidation is one of the required tests. Also, the hydrolytic and thermal stability are required. An ideal stability-indicating method is one that quantifies the drug per se and also resolves its degradation products. Nowadays, HPTLC is becoming a routine analytical technique due to its advantages [8–12]. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis. Mobile phase having pH 8 and above can be employed. Suspensions, dirty or turbid samples can be directly applied. It facilitates automated application and scanning in situ. HPTLC facilitates repeated detection (scanning) of the chromatogram with the same or different parameters. Simultaneous assay of several components in a multicomponent

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formulation is possible. The aim of this work is to develop an accurate, specific, repeatable and stability-indicating method for the determination of imatinib mesylate in the presence of its degradation products as per ICH guidelines [13,14].

2. Experimental

2.1. Materials

Imatinib mesylate was a gift sample from Natco Pharmaceuticals, Hyderabad, India. All chemicals and reagents used were of analytical grade and purchased from Qualigens Fine Chemicals, Mumbai, India.

2.2. HPTLC instrumentation

The samples were spotted in the form of bands of width 6 mm with a Camag microliter syringe on precoated silica gel aluminium Plate 60F-254 (20 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat IV (Switzerland). A constant application rate of 100 nl/s was employed and space between two bands was 8 mm. The slit dimension was kept 5 mm × 0.45 mm micro, 5 mm/s scanning speed was employed. The mobile phase consisted of chloroform:methanol (6:4, v/v). Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature. The length of chromatogram run was approximately 70 mm. Subsequent to the development; TLC plate was dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 276 nm. The source of radiation utilized was deuterium lamp.

2.3. Calibration curve of imatinib mesylate

A stock solution of imatinib mesylate (1000 μg/ml) was prepared in methanol. Different volume of stock solution, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml was taken and volume made up to 10 ml by methanol, to made 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μg/ml solution, respectively. The 10 μl of each above solutions were spotted in three replicate on TLC plate to obtain concentration of 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 ng per spot of imatinib mesylate, respectively. The data of peak area versus drug concentration were treated by linear least square regression.

2.4. Method validation

2.4.1. Precision

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (500 ng per spot of imatinib mesylate). The intra- and inter-day variation for the determination of imatinib mesylate was carried out at three different concentration levels of 300, 500 and 700 ng per spot.

2.4.2. Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition of chloroform:methanol (5.5:4.5 and 6.5:3.5, v/v) were tried and chromatograms were run. The amount of mobile phase, temperature and relative humidity was varied in the range of ±5%. The plates were prewashed by methanol and activated at 60 ± 5 °C for 2, 5 and 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, 40 and 60 min. Robustness of the method was done at three different concentration levels: 300, 500 and 700 ng per spot.

2.4.3. Limit of detection and limit of quantification

In order to determine detection and quantification limit, imatinib mesylate concentrations in the lower part of the linear range of the calibration curve were used. Imatinib mesylate solutions of 10, 12, 14, 16, 18 and 20 μg/ml were prepared and applied in triplicate (10 μl each). The amount of imatinib mesylate by spot versus average response (peak area) was graphed and the equation for this was determined. The standard deviations (S.D.) of responses were calculated. The average of standard deviations was calculated (A.S.D.). Detection limit was calculated by (3.3 × A.S.D.)/*b* and quantification limit was calculated by (10 × A.S.D.)/*b*, where "*b*" corresponds to the slope obtained in the linearity study of method.

2.4.4. Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for imatinib mesylate in sample was confirmed by comparing the R_f values and spectra of the spot with that of standard. The peak purity of imatinib mesylate was assessed by comparing the spectra at three different levels, i.e., peak start (*S*), peak apex (*M*) and peak end (*E*) positions of the spot.

2.4.5. Recovery studies

The analysed samples were spiked with extra 50, 100 and 150% of the standard imatinib mesylate and the mixture were analysed by the proposed method. At each level of the amount, six determinations were performed. This was done to check the recovery of the drug at different levels in the formulations.

2.5. Analysis of imatinib mesylate in prepared formulation

To determine the concentration of imatinib mesylate in capsules (labeled claim: 100 mg per capsule), the contents of 20 capsules were weighed, their mean weight determined and they were finely powdered. The powder equivalent to 10 mg of imatinib mesylate was weighed. The drug from the powder was extracted with methanol. To ensure complete extraction of the drug, it was sonicated for 30 min and the volume was made up to 10 ml. The resulting solution was centrifuged at 3000 rpm for 5 min and supernatant was analysed for drug content. The 0.5 ml was taken and volume made up to 10 ml by methanol, to made 50 μg/ml solution. The 10 μl of the above solution (500 ng per spot) was applied on TLC plate followed by development and

scanning as described in Section 2.2. The analysis was repeated in triplicate. The possibility of excipient interferences in the analysis was studied.

2.6. Forced degradation of imatinib mesylate

2.6.1. Acid and base induced degradation

The 10 mg of imatinib mesylate was separately dissolved in 10 ml of methanolic solution of 0.1 M HCl and 1 M NaOH. These solutions were kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. The 1 ml of above solutions was taken and neutralized, then diluted up to 10 ml with methanol. The resultant solution were applied on TLC plate in triplicate (10 μl each, i.e. 1000 ng per spot). The chromatograms were run as described in Section 2.2.

2.6.2. Hydrogen peroxide-induced degradation

The 10 mg of imatinib mesylate was separately dissolved in 10 ml of methanolic solution of hydrogen peroxide (3.0%, v/v). The solution was kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. The 1 ml of above solution was taken and diluted up to 10 ml with methanol. The resultant solution was applied on TLC plate in triplicate (10 μl each, i.e. 1000 ng per spot). The chromatograms were run as described in Section 2.2.

2.6.3. Dry heat degradation product

The powdered drug was stored at 55 °C for 3 h under dry heat condition showed significant degradation. The degraded products were resolved from the standard.

In all degradation studies, the average peak areas of imatinib mesylate after application (1000 ng per spot) of three replicates were obtained.

3. Results and discussion

3.1. Development of optimum mobile phase

TLC procedure was optimized with a view to developing a stability-indicating assay method. Initially, chloroform:methanol (7:3, v/v) gave good resolution with R_f value of 0.53 for imatinib mesylate but typical peak nature was missing. Finally, the mobile phase consisting of chloroform:methanol (6:4, v/v) gave a sharp and well defined peak at R_f value of 0.53 (Fig. 1). Well-defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature.

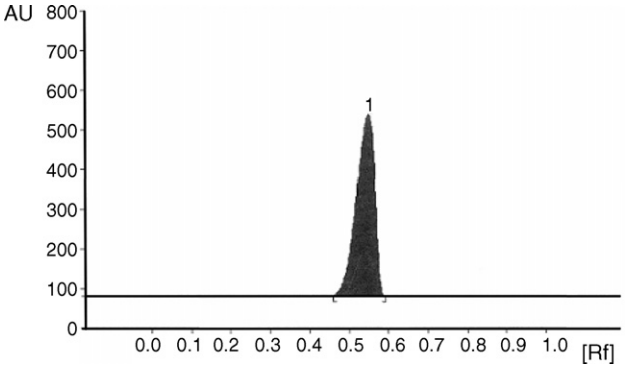

Fig. 1. A typical HPTLC chromatogram of imatinib mesylate (R_f = 0.53).

Table 1
Linear regression data for the calibration curves[a]

Linearity range (ng per spot)	100–1000
$r^2 \pm$ S.D.	0.9966 ± 0.0013
Slope ± S.D.	164.85 ± 0.72
Confidence limit of slope[b]	164.03–165.66
Intercept ± S.D.	1168.3 ± 8.26
Confidence limit of intercept[b]	1158.95–1177.64

[a] n = 3.
[b] 95% confidence limit.

3.2. Calibration curves

The linear regression data for the calibration curves (n = 3) as shown in Table 1 showed a good linear relationship over the concentration range 100–1000 ng per spot with respect to peak area.

No significant difference was observed in the slopes of standard curves (ANOVA, $P > 0.05$)

3.3. Validation of the method

3.3.1. Precision

The repeatability of sample application and measurement of peak area were expressed in the terms of %R.S.D. and results are depicted in Table 2, which revealed intra- and inter-day variation of imatinib mesylate at three different concentration levels of 300, 500 and 700 ng per spot.

Table 2
Intra- and inter-day precision of HPTLC method[a]

Amount (ng per spot)	Intra-day precision				Inter-day precision			
	Mean area	S.D.	%R.S.D.	S.E.[b]	Mean area	S.D.	%R.S.D.	S.E.[b]
300	5960.18	35.21	0.59	14.37	5882.36	54.12	0.92	22.09
500	9522.25	42.56	0.45	17.37	9488.92	62.22	0.66	25.40
700	13311.50	54.24	0.41	22.14	13194.29	84.81	0.64	34.62

[a] n = 6.
[b] Standard error.

Table 3
Robustness of the method[a]

Parameter	S.D.[b] of peak area	%R.S.D.[b]
Mobile phase composition	1.23	0.98
Amount of mobile phase	1.06	0.85
Temperature	0.92	0.68
Relative humidity	0.88	0.56
Plate pretreatment	0.59	0.41
Time from spotting to chromatography	0.41	0.36
Time from chromatography to scanning	0.36	0.31

[a] $n = 6$.
[b] Average of three concentrations: 300, 500 and 700 ng per spot.

3.3.2. Robustness of the method

The standard deviation of peak areas was calculated for each parameter and %R.S.D. was found to be less than 2%. The low values of %R.S.D. values as indicated are shown in Table 3 indicated robustness of the method.

3.3.3. LOD and LOQ

The calibration curve in this study was plotted between amount of analyte versus average response (peak area) and the regression equation was obtained ($Y = 154.27X + 1313.9$) with a regression coefficient of 0.9964. Detection limit and quantification limit was calculated by the method as described in Section 2.4.3 and found 6.51 and 19.72 ng, respectively. However, by experiment, we got LOD and LOQ was 10 and 30 ng, respectively. This indicates the adequate sensitivity of the method.

3.3.4. Specificity

The peak purity of imatinib mesylate was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot, i.e., $r^2\ (S, M) = 0.9998$ and $r^2\ (M, E) = 0.9988$. Good correlation ($r^2 = 0.9989$) was also obtained between standard and sample spectra of imatinib mesylate.

3.3.5. Recovery studies

The proposed method when used for extraction and subsequent estimation of imatinib mesylate from pharmaceutical dosage forms after spiking with 50, 100 and 150% of additional drug afforded recovery of 98–102% as listed in Table 4.

The data of summary of validation parameters were listed in Table 5.

Table 4
Recovery studies[a]

Excess drug added to[b] the analyte (%)	Amount recovered (mg)	Recovery (%)	%R.S.D.	S.E.
0	101.21	101.21	0.42	0.34
50	150.93	100.62	0.68	0.55
100	198.68	99.34	0.72	0.57
150	247.15	98.86	0.48	0.38

[a] $n = 6$.
[b] Matrix containing 100 mg drug.

Table 5
Summary of validation parameters

Parameter	Data
Linearity range (ng per spot)	100–1000
Correlation coefficient	0.9966 ± 0.0013
Limit of detection (ng per spot)	10
Limit of quantitation (ng per spot)	30
Recovery ($n = 6$)	100.01 ± 1.32
Precision (%R.S.D.)	
Repeatability of application ($n = 6$)	0.35
Repeatability of measurement ($n = 6$)	0.16
Inter-day ($n = 6$)	0.74
Intra-day ($n = 6$)	0.48
Robustness	Robust
Specificity	Specific

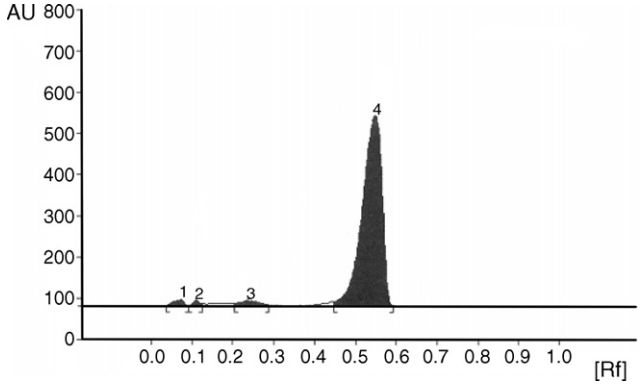

Fig. 2. HPTLC chromatogram of acid degraded imatinib mesylate.

3.4. Analysis of prepared formulation

A single spot of R_f 0.53 was observed in chromatogram of the imatinib mesylate samples extracted from capsules. There was no interference from the excipients commonly present in the capsules. The imatinib mesylate content was found to be 99.2% with a %R.S.D. of 0.63. It may therefore be inferred that degradation of imatinib mesylate had not occurred in the formulation that were analysed by this method. The low %R.S.D. value indicated the suitability of this method for routine analysis of imatinib mesylate in pharmaceutical dosage forms.

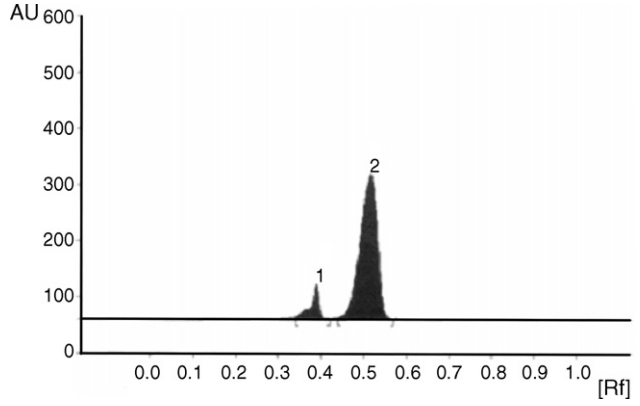

Fig. 3. HPTLC chromatogram of base degraded imatinib mesylate.

Table 6
Forced degradation of imatinib mesylate

Serial number	Sample exposure condition	Number of degradation products (R_f value)	Imatinib mesylate remained (ng/1000 ng) (±S.D, $n = 3$)	S.E.	Recovery (%)
1	0.1 M HCl, 8 h, RT	3 (0.8, 0.11, 0.24)	920.21 (±1.52)	0.88	92.02
2	1 M NaOH, 8 h, RT	1 (0.38)	721.86 (±4.26)	2.46	72.19
3	3% H_2O_2, 8 h, RT	2 (0.5, 0.8)	811.46 (±3.91)	2.26	81.15
4	Heat, 3 h, 55 °C	2 (0.38, 0.7)	775.26 (±5.98)	3.45	77.53

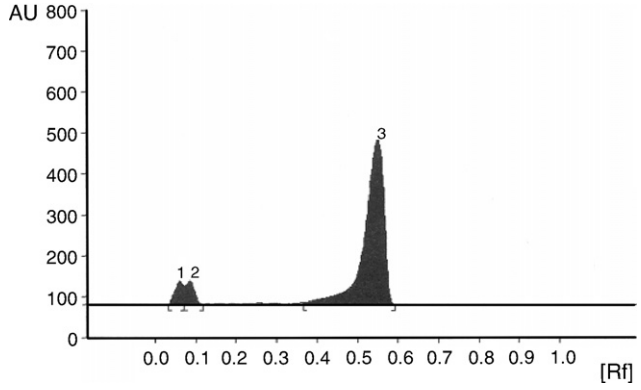

Fig. 4. HPTLC chromatogram of hydrogen peroxide degraded imatinib mesylate.

3.5. Stability-indicating property

The chromatogram of samples degraded with acid, base, hydrogen peroxide and heat showed well separated spots of pure imatinib mesylate as well as some additional peaks at different R_f values. The spots of degraded product were well resolved from the drug spot as shown in Figs. 2–5. The number of degradation product with their R_f values, content of imatinib mesylate remained, and percentage recovery were calculated and listed in Table 6.

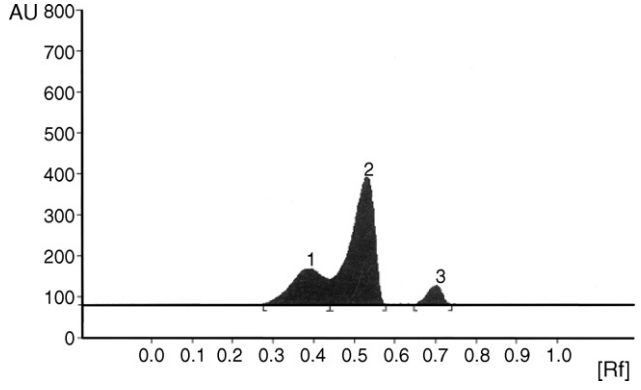

Fig. 5. HPTLC chromatogram of heat degraded imatinib mesylate.

4. Conclusion

The developed HPTLC technique is precise, specific, accurate and stability indicating. The developed method was validated based on ICH guidelines [12]. Statistical analysis proves that the method is repeatable and selective for the analysis of imatinib mesylate as bulk drug and in pharmaceutical formulations. The method can be used to determine the purity of the drug available from the various sources by detecting the related impurities. It may be extended to study the degradation kinetics of imatinib mesylate and for its estimation in plasma and other biological fluids. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

References

[1] R.G. Maki, Curr. Treat. Options Gastroenterol. 7 (2004) 13–17.
[2] I.M. Adcock, K.F. Chung, G. Caramori, K. Ito, Eur. J. Pharmacol. 533 (2006) 118–132.
[3] V.V. Vivekanand, S. Rao, G. Vaidyanathan, N.M. Sekher, S.A. Kelkar, P.R. Puranik, J. Pharm. Biomed. Anal. 33 (2003) 879–889.
[4] T. Velapandian, R. Mathur, N.K. Agarwal, B. Arora, L. Kumar, S.K. Gupta, J. Chromatogr. B 804 (2004) 431–434.
[5] R. Bakhtiar, L. Khemani, M. Hayes, T. Bedman, F. Tse, J. Pharm. Biomed. Anal. 28 (2002) 1183–1194.
[6] R.A. Parise, R.K. Ramanathan, M.J. Hayes, M.J. Egorin, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 79 (2003) 39–44.
[7] ICH, International Conference on Harmonization, Geneva, October, 1993.
[8] P.D. Sethi, High Performance Thin Layer Chromatography, Quantitative Analysis of Pharmaceutical Formulations, CBS Publishers, 1996.
[9] S.P. Kulkarni, P.D. Amin, J. Pharm. Biomed. Anal. 23 (2000) 983–987.
[10] S.O. Thoppil, R.M. Cardoza, P.D. Amin, J. Pharm. Biomed. Anal. 25 (2001) 15–20.
[11] S.N. Makhija, P.R. Vavia, J. Pharm. Biomed. Anal. 25 (2001) 663–667.
[12] D. Ivanovic, M. Medenica, B. Jancic, A. Malenovic, J. Chromatogr. B 800 (2004) 253–258.
[13] ICH, Proceedings of the International Conference on Harmonization, Geneva, October, 1994.
[14] M. Bakshi, S. Singh, J. Pharm. Biomed. Anal. 28 (2002) 1011–1040.