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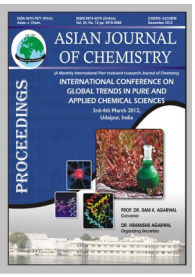

Kinetic Spectrophotometric Methods for the Determination of Imatinib Mesylate in Pure and Tablet Dosage Form†

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Simple and sensitive kinetic spectrophotometric methods were established for the determination of imatinib mesylate in pure and in its pharmaceutical dosage forms using alkaline potassium permanganate as an oxidizing agent. The methods involve determination of imatinib mesylate by kinetic studies of its oxidation at room temperature for a fixed time of 5 min. The absorbance of the green coloured manganate ions was measured at 610 nm. Alternatively, the decrease in the absorbance of permanganate upon addition of the studied drug was also measured at 525 nm. The absorbance-concentration plots were rectilinear over the ranges of 1-34 and 2-20 µg/mL for the two methods respectively. The different experimental parameters affecting the reaction were carefully studied and optimized. The stoichiometry of the reaction was determined adopting the limiting logarithmic method. The analytical performance of the proposed methods was fully validated and the results were satisfactory. The proposed methods have a great value in their application to the analysis of imatinib mesylate in quality control laboratories.

Key Words: Imatinib mesylate, Kinetic spectrophotometry, Fixed time method, Validation, Pharmaceutical preparations.

INTRODUCTION

Imatinib mesylate or gleevec, is chemically designated as 4-[(4-methyl-1-piperazinyl)methyl]-*N*-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide methanesulfonate. It is a chemotherapy drug indicated for the treatment of patients with chronic myeloid leukemia (CML) in blast crisis, accelerated phase, or in chronic phase after failure of interferon-alpha therapy[1].

Several analytical methods have been reported for the determination of imatinib mesylate in pure form, in pharmaceuticals and in biological fluids. Most of the reported methods are chromatographic methods and no official method has been reported for the determination of imatinib mesylate. The reported methods include: high performance liquid chromatography with ultraviolet detection (HPLC/UV)[2-6], ion pairing reversed phase high performance liquid chromatography[7], liquid chromatography-electrospray mass spectrometry (LC/ESI/MS)[8], liquid chromatography tandam mass spectrometry (LC/MS/MS][9], stability indicating reversed phase liquid chromatography[10], stability indicating high performance thin layer liquid chromatography (HPTLC)[11], adsorptive stripping square wave voltammetry[12-14], UV-spectrophotometry[15-17] and visible spectrophotometry[18,19].

Kinetic spectrophotometric methods are becoming of a great interest in the pharmaceutical analysis[20]. The aim of the present work was to develop simple and sensitive kinetic spectrophotometric methods for the determination of imatinib in bulk and in its pharmaceutical preparations using alkaline potassium permanganate as an oxidizing agent. The methods involve determination of imatinib by kinetic studies of its oxidation at room temperature for a fixed time of 5 min. The absorbance of the coloured manganate ions was measured at 610 nm. Alternatively, the decrease in the absorbance of permanganate upon addition of the studied drug was also measured at 525 nm. The methods were successfully applied for the determination of this drug in its dosage forms.

EXPERIMENTAL

Spectrophotometric measurements were performed using an UV-VIS spectrophotometer model ultrospec 2100-Pro (Biochrom, England) with matched 1 cm quartz cells.

Pure imatinib mesylate was kindly supplied by Novartis Pharma AG, Basel, Switzerland. The commercial glivec tablets (B. No. S0093) are labeled to contain 100 mg imatinib mesylate per tablet.

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The following reagents were used: Potassium permanganate (BDH, UK) was 1.0×10^{-2} M aqueous solution. Sodium hydroxide (Winlab, Middlesex, UK) was 1 M aqueous solution.

General analytical procedure: A stock solution containing 1 mg/mL of imatinib mesylate was prepared in distilled water. Working standard solutions were prepared from stock solution by further dilution with distilled water. Aliquot solutions containing 10-340 µg (for 610 nm) or 20-200 µg (for 525 nm) of imatinib mesylate were transferred into a series of 10 mL volumetric flasks; 3 mL of 1.0 M NaOH (for 610 nm) or 1.5 mL of 1 M NaOH (for 525 nm), followed by 3 mL of 1×10^{-2} M $KMnO_4$ (for 610 nm) or 0.4 mL of 1×10^{-2} M $KMnO_4$ (for 525 nm) were added. The mixtures were shaken well and completed to volume with distilled water. The increase in the absorbance at 610 nm or the decrease at 525 nm was measured after 5 min at ambient temperature (25 ºC) against an appropriate blank prepared simultaneously. The calibration graphs were obtained by plotting the absorbance (A) at 610 nm or the difference in the absorbance (ΔA) at 525 nm *versus* concentration of the drug in µg/mL. Alternatively, the corresponding regression equations were calculated.

Analysis of tablets: An accurately weighed amount of 10 powdered tablets equivalent to 10 mg of imatinib mesylate was transferred into a 100 mL volumetric flask and completed to volume with distilled water. The flask with its contents was sonicated for 0.5 h and then filtered. The above procedure was followed. The nominal content was calculated either from the previously plotted calibration graphs or using the corresponding regression equations.

RESULTS AND DISCUSSION

The reaction between imatinib mesylate and $KMnO_4$ in alkaline solution yields a green colour as a result of manganate ions. As the intensity of colour increases with time, it was deemed useful to elaborate kinetically based methods for the determination of imatinib mesylate.

Spectral studies: The aqueous solution of imatinib mesylate shows one absorption band at 256 nm while that of alkaline potassium permanganate solution exhibits two absorption maxima at 525 and 545 nm. The course of the reaction starts on the addition of aqueous alkaline potassium permanganate to the solution of imatinib mesylate resulting in the formation of new band peaking at 610 nm. This band is attributed to the formation of manganate ions in the presence of the drug. The intensity of the green coloured solution increased with time owing to the formation of MnO_4^{2-}, whereas the absorbance of the solution at 525 nm decreased as the reaction proceeded due to the disappearance of MnO_4^-. These facts were used to develop kinetically based spectrophotometric methods for the determination of imatinib mesylate.

Optimization of the reaction conditions: The factors affecting the reaction conditions were carefully studied and optimized. Such factors were changed individually while the others were kept constant. These factors were concentrations of $KMnO_4$ and alkalinity. The effect of $KMnO_4$ concentration on the absorbance of the reaction product was studied at 610 nm using different volumes (0.2-4 mL) of 1×10^{-2} M $KMnO_4$. It was found that maximum absorbance was attained using 2.5 mL, after which $KMnO_4$ has no effect on the absorbance. Thus 3 mL of 1×10^{-2} M $KMnO_4$ was used for further studies at 610 nm. Also, 0.4 mL of 1×10^{-2} M was sufficient for measuring the decrease in the absorbance at 525 nm. The effect of NaOH concentration on the absorbance of the reaction product was investigated using different volumes (0.2-4 mL) of 1 M NaOH. It was found that increasing the volume of 1 M NaOH would increase A or ΔA of the reaction up to 2.5 mL at 610 or 1mL at 525 nm, after that the absorbance was constant. Thus 3 mL of 1.0 M NaOH (at 610 nm) or 1.5 mL of 1 M NaOH (at 525 nm) were used for further studies.

Stoichiometry of the reaction: The stoichiometry of the reaction was studied adopting the limiting logarithmic method[21]. A plot of log absorbance *vs.* log [$KMnO_4$] and *vs.* log [imatinib mesylate] gave straight lines, with slope values of 0.97 and 0.87 respectively. Therefore, the reaction proceeds in ratio of 1:1 ($KMnO_4$ to drug).

Study of the kinetic parameters: Under the optimized experimental conditions, the determination of imatinib mesylate was carried out in an excess of $KMnO_4$ and NaOH with respect to the initial concentration of imatinib mesylate. As a result, pseudo zero order reaction conditions were obtained with respect to their concentrations. Therefore, on the basis of the kinetic investigation, the kinetic equation for the oxidation of imatinib mesylate by $KMnO_4$ in alkaline medium is written as:

$$\nu = k`C^n$$

where, ν is the reaction rate, k` is pseudo rate constant, C is the molar concentration of imatinib mesylate and n is the order of the reaction. The logarithmic form of equation may be written as: $\log \nu = \log k` + n \log C$.

The initial rates of reaction were evaluated at different concentrations of imatinib mesylate 0.339×10^{-4} - 4.239×10^{-4} M (at 610 nm) and 0.339×10^{-4} - 3.39×10^{-4} M (at 525 nm) by measuring the slopes of the initial tangent to the absorbance-time curves (at 610 and 525 nm) (Fig. 1). The logarithms of reaction rates were plotted as a function of logarithms of imatinib concentration at 610 and 525 nm. Regression analysis of the data using the method of least squares[22] gave the following equations:

At 610 nm: $\log \nu = 0.6197 + 1.028 \log C$ (r = 0.9995)

At 525 nm: $\log \nu = 0.6203 + 1.103 \log C$ (r = 0.9989)

Hence k`= 4.16 s^{-1}, 4.17 s^{-1} and n = 1.03, 1.10 (*ca.* 1) respectively, thus the reaction was pseudo first order with respect to imatinib.

Initial rate method: In this method, initial reaction rates were determined from the slopes of tangents of absorbance-time curves at 610 and 525 nm (Fig. 1). The calibration curves were constructed by plotting the initial rate (ν) *vs.* the concentration of imatinib. Linear relationships were obtained over the concentration range of 0.339×10^{-4} - 4.239×10^{-4} M (2-25 µg/mL) at 610 nm and 0.339×10^{-4} - 3.390×10^{-4} M (2-20 µg/mL) at 525 nm. Regression analysis of the data using the method of least squares[22] gave the following equations:

At 610 nm: $\nu = -3.130 \times 10^{-6} + 3.312\ C$ (r = 0.9996)

At 525 nm: $\nu = -1.645 \times 10^{-5} + 1.835\ C$ (r = 0.9997)

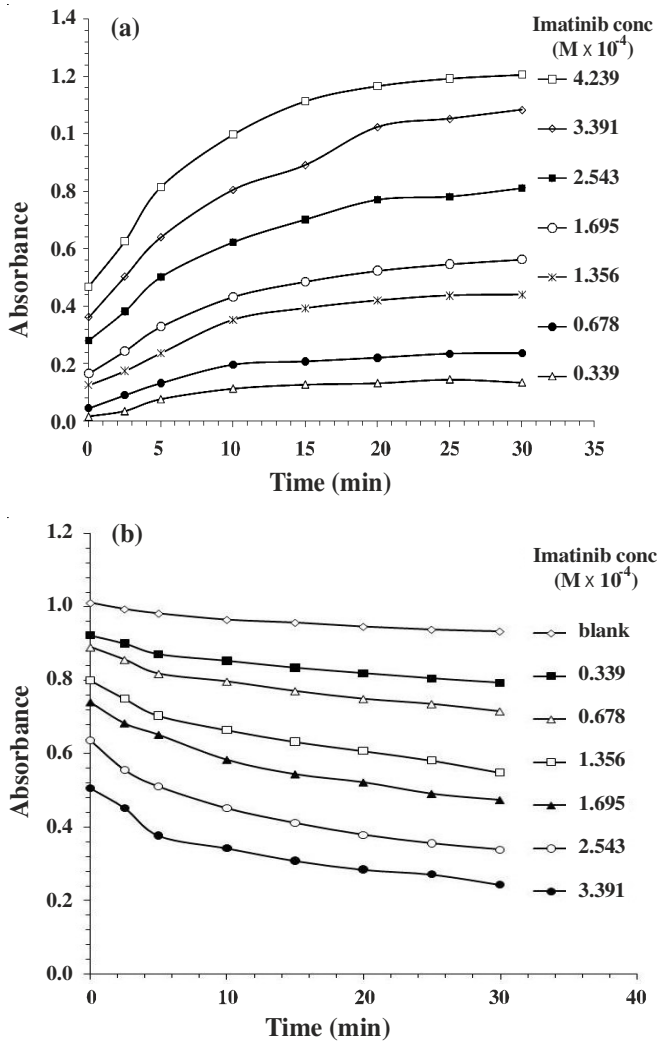

Fig. 1. Absorbance-time curves for the reaction of different concentrations of imatinib mesylate with alkaline potassium permanganate. (A) at 610 nm, (B) at 525 nm

Fixed time method: At a pre-selected fixed time, which was accurately determined, the absorbance was measured at 610 nm and ΔA was measured at 525 nm. Calibration graphs were obtained by plotting the absorbance at 610 nm or ΔA at 525 nm *vs*. initial concentrations of imatinib at fixed time of 2.5, 5, 10, 15, 20, 25, 30 min were established. The regression equations, correlation coefficients and standard deviation of slope and intercept are given in Table-1. It is clear that the most acceptable values of the correlation coefficient (r) and the intercept were obtained at a fixed time of 5 min, which was chosen as the most suitable time interval for the measurement. Calibration graphs for the determination of imatinib was obtained over the concentration ranges of 1.0-34.0 μg/mL and 2.0-20.0 μg/mL by the fixed time method at 610 and 525 nm, respectively.

Fixed concentration method (Fixed reaction extent): Reaction times were determined for different concentrations of imatinib to reach a specific absorbance at 610 nm or ΔA at 525 nm. A pre-selected value (0.30) was fixed and the time was measured in seconds. Calibration graphs were obtained by plotting the reciprocal of time (1/t) *vs*. initial concentration of drug (C). Straight lines were obtained over the concentration ranges of 2.0-10.0 μg/mL and 4.0-10.0 μg/mL at 610 and 525 nm respectively.

In conclusion, the fixed concentration method has poor linearity, poor accuracy and poor sensitivity than the initial rate and fixed time methods. Although, the initial rate method gave an acceptable value of correlation coefficient but it possess serious experimental difficulty in accurately determining the initial rate. Also the range of imatinib mesylate concentration giving the most acceptable calibration graph is very limited in the fixed concentration methods. Thus, the fixed time method was chosen for the determination of imatinib mesylate in pure form and in its tablets. This method was further validated for linearity, accuracy, intra-day and inter-day precision,

TABLE - 1
ANALYTICAL PARAMETERS FOR THE PROPOSED FIXED TIME SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF IMATINIB MESYLATE USING ALKALINE POTASSIUM PERMANGANATE AT 610 AND 525 nm

	Time (min)	Linear range (μg/mL)	Regression equation A = a + b C*	Correlation coefficient (r)	Standard deviation of slope (S_b)	Standard deviation of intercept (S_a)	LOD (μg/mL)	LOQ (μg/mL)
At 610 nm	2.5	1.0– 34.0	A = -0.0198 + 0.0261 C	0.9996	2.67×10^{-4}	0.0052	0.65	1.99
	5	1.0– 34.0	A = 0.0091 + 0.0319 C	0.9999	1.37×10^{-4}	0.0026	0.27	0.82
	10	1.0– 30.0	A = 0.0412 + 0.0386 C	0.9998	3.02×10^{-4}	0.0051	0.44	1.32
	15	1.0 – 25.0	A = 0.0427 + 0.0429 C	0.9996	4.69×10^{-4}	0.0063	0.48	1.46
	20	1.0– 20.0	A = 0.0246 + 0.0497 C	0.9998	4.19×10^{-4}	0.0045	0.29	0.91
	25	1.0– 15.0	A = 0.0361 + 0.0501 C	0.9994	7.96×10^{-4}	0.0066	0.43	1.31
	30	1.0 –15.0	A = 0.0375 + 0.0515 C	0.9993	9.16×10^{-4}	0.0076	0.48	1.47

*A = absorbance, C = concentration in μg/mL

	Time (min)	Linear range (μg/mL)	Regression equation ΔA = a + b C*	Correlation coefficient (r)	Standard deviation of slope (S_b)	Standard deviation of intercept (S_a)	LOD (μg/mL)	LOQ (μg/mL)
At 525 nm	2.5	2.0– 20.0	ΔA = 0.0432 + 0.0251 C	r = 0.9996	3.07×10^{-4}	0.0035	0.46	1.39
	5	2.0– 20.0	ΔA = 0.0557 + 0.0276 C	r = 0.9999	1.45×10^{-4}	0.0016	0.19	0.58
	10	2.0– 20.0	ΔA = 0.0731 + 0.0293 C	r = 0.9994	5.04×10^{-4}	0.0058	0.65	1.98
	15	2.0– 20.0	ΔA = 0.0663 + 0.0316 C	r = 0.9998	3.16×10^{-4}	0.0036	0.37	1.14
	20	2.0– 20.0	ΔA = 0.0694 + 0.0328 C	r = 0.9996	4.44×10^{-4}	0.0052	0.52	1.58
	25	2.0– 20.0	ΔA = 0.0953 + 0.0320 C	r = 0.9995	4.68×10^{-4}	0.0054	0.56	1.69
	30	2.0– 15.0	ΔA = 0.0774 + 0.0360 C	r = 0.9996	5.71×10^{-4}	0.0052	0.48	1.44

*ΔA = difference in absorbance, C = concentration in μg/mL

repeatability, robustness and ruggedness accordance with ICH guidelines[23] and the results were satisfactory.

Application of the proposed methods: In order to evaluate the analytical usefulness of the proposed kinetic spectrophotometric methods, imatinib mesylate was determined in pure and in its tablets. For pure form, the % found was 100.16 ± 1.06 (at 610 nm) and 99.90 ± 1.04 (at 525 nm). The results obtained were in good agreement with those obtained by the published spectrophotometric method[17]. Table-2 shows the results of analysis of imatinib in its tablets. Statistical analysis[22] of the results obtained by the proposed and the comparison methods shows no significant difference between the two methods as regards to accuracy (*t*-test) and precision (*F*-test).

TABLE- 2
ANALYSIS OF IMATINIB MESYLATE IN COMMERCIAL TABLETS BY THE PUBLISHED METHOD AND THE PROPOSED FIXED TIME SPECTROPHOTOMETRIC METHOD USING ALKALINE POTASSIUM PERMANGANATE

Methods		Concentration taken (µg/mL)	Found[a] (%)	
			Proposed method	Comparison method[17]
At 610 nm	Glivec tablets (100 mg/tablet)[b]	2.0	98.92	101.67
		5.0	100.90	100.62
		10.0	99.81	100.00
		12.0	102.13	102.20
		20.0	100.40	
Mean ± S.D.			100.43 ± 1.20	101.12 ± 1.00
Student's *t*-value			0.920 (2.365)[c]	
Variance ratio *F-test*			1.44 (9.12)[c]	
At 525 nm	Glivec tablets (100 mg/tablet)[b]	2.0	98.62	101.67
		5.0	101.76	100.62
		10.0	101.96	100.00
		12.0	98.80	102.20
		20.0	101.37	
Mean ± S.D.			100.50 ± 1.65	101.12 ±1.00
Student's *t*-value			0.615 (2.365)[c]	
Variance ratio *F-test*			2.72 (9.12)[c]	

[a]Each result is the average of three separate determinations; [b]Products of Novartis Pharma AG, Basel, Switzerland; [c]The figures between parentheses are the tabulated values of *t* and *F* at P = 0.05[22].

Conclusion

The present study described, for the first time, simple and sensitive kinetic spectrophotometric methods for the determination of imatinib mesylate in its dosage forms. The proposed fixed time method can be easily applied as it does not require elaborate treatment of the samples and/or tedious procedure for the analysis. Furthermore, the proposed methods do not require expensive instruments and/or critical to analytical reagents. These advantages give the proposed methods a great value and encourage their application to the analysis of imatinib mesylate in quality control laboratories.

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REFERENCES

1. C. Gambacorti-Passerini, R. Barni, P. Le Coutre, M. Zucchetti, G. Cabrita, L. Cleris, F. Rossi, E. Gianazza, J. Brueggen, R. Cozens, P. Pioltelli, E. Pogliani, G. Corneo, F. Formelli and M. D'Incalci, *J. Nat. Cancer Inst*., **44**, 433 (2000).
2. A. Awidi, I.I. Salem, N. Najib, R. Mefleh and B. Tarawneh, *Leukemia Res.*, **34**, 714 (2010).
3. M. Rosasco, M. Moyano, M. Pizzorno and A. Segall, *J. Liq. Chromatogr. Rel. Technol.*, **28**, 3283 (2005).
4. T. Velpandian, R. Mathur, N.K. Agarwal, B. Arora, L. Kumar and S.K. Gupta, *J. Chromatogr. B*, **804**, 431 (2004).
5. N. Widmer, A. Beguin, B. Rochat, T. Buclin, T. Kovacsovics, M.A. Duchosal, S. Leyvraz, A. Rosselet, J. Biollaz and L.A. Decosterd, *J. Chromatogr. B*, **803**, 285 (2004).
6. D. Ivanovic, M. Medenica, B. Jancic and A. Malenovic, *J. Chromatogr. B*, **800**, 253 (2004).
7. R.L. Oostendorp, J.H. Beijnen, J.H.M. Schellens and O. Van Tellingen, *Biomed. Chromatogr.*, **21**, 747 (2007).
8. I. Solassol, F. Bressolle, L. Philibert, V. Charasson, C. Astre and F. Pinguet, *J. Liq. Chromatogr. Rel. Technol.*, **29**, 2957 (2006).
9. P. Karine, D.D. Stephane, E. Teilhet, N. Moore, P. Berthaud, F.X. Mahon and M. Molimard, *Therap. Drug Monit.*, **27**, 634 (2005).
10. G. Bende, S. Kollipara, V. Kolachina and R. Saha, *Chromatographia*, **66**, 859 (2007).
11. N. Vadera, G. Subramanian and P. Musmade, *J. Pharm. Biomed. Anal.*, **43**, 722 (2007).
12. J. Rodriguez, J.J. Berzas, G. Castaneda and N. Rodriguez, *Talanta*, **66**, 202 (2005).
13. V.C. Diculescu, M. Vivan and A.M.O. Bretta, *Electroanalysis*, **18**, 1800 (2006).
14. E. Hammam, H.S. El-Desoky, A. Tawfik and M.M. Ghoneim, *Can. J. Chem.*, **82**, 1203 (2004).
15. G. Bende, S. Kollipara, V. Sekar and R. Saha, *Pharmazie*, **63**, 641 (2008).
16. D.G. Sankar, P.V.M. Latha and M.V. Krishna, *Asian J. Chem.*, **18**, 1543 (2006),
17. J.K. Raja, V.D. Sundar, A.R. Magesh, S.N. Kumar and M.D. Dhanaraju, *Int. J. Pharm. Technol.*, **2**, 490 (2010).
18. V.M. Balaram, J.V. Rao, M.M.A. Khan, J.V.C. Sharma and K. Anupama, *Asian J. Chem.*, **21**, 5241 (2009).
19. D.G. Sankar, P.V.M. Latha and M.V. Krishna, *Asian J. Chem.*, **18**, 1531 (2006).
20. I.A. Darwish, *J. Anal. Chem.*, **551**, 222 (2005).
21. J. Rose, Advanced Physicochemical Experiments Pitman, London, p. 348 (1964).
22. R. Caulcutt and R. Boddy, Statistics for analytical Chemists, Chapman and Hall, London (1983).
23. ICH Technical Requirements for Registration of Pharmaceuticals for Human Use, Complementary Guidelines on Methodology. Washington DC, p. 13 (1996).