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UV-spectrophotometric determination of imatinib mesylate and its application in solubility studies

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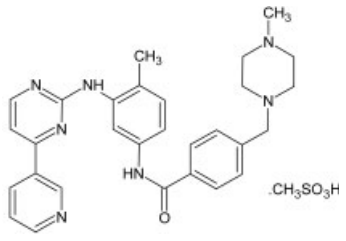
A new, simple and sensitive UV-spectrophotometric method was developed for the determination of imatinib mesylate in bulk and pharmaceutical formulations (tablets and nanoparticles). The developed spectroscopic method was validated for selectivity, linearity and range, precision, accuracy and sensitivity. The method has demonstrated excellent linearity over the range of 2.5–25 μg/mL with regression equation: absorbance (AU) = 0.047 × concentration (μg/mL) + 0.008 and $r^2 = 0.9998$. The developed method demonstrated consistent high recoveries (99–102%) and low relative standard deviation (< 5%) at 285 nm. Moreover, the method was found to be highly sensitive with low limit of detection (0.57 μg/mL) and limit of quantitation (1.71 μg/mL). The apparent molar absorptivity and Sandell's sensitivity was found to be 2.75×10^3 L/M cm and 2.15 μg/cm^2 respectively. The validated method was successfully employed for the drug content analysis from tablets and nanoparticles preparations. Additionally, the method was successfully employed for pH metric solubility analysis of the drug.

1. Introduction

Imatinib mesylate (IM) represents a novel class of rationally designed targeted chemotherapeutic agents and is considered as a paradigm for cancer chemotherapy (Druker 2002; Mauro et al. 2002; Druker 2004). Protein tyrosine kinase is a family of signaling proteins involved in a variety of important cellular processes such as cell proliferation, migration, differentiation and survival. Deregulated protein tyrosine kinases is implicated in several human malignancies (Arora and Scholar 2005; Arslan et al. 2006). Since its accelerated approval for first-line treatment of Philadelphia chromosome positive chronic myeloid leukemia, IM has been found to be useful in selective inhibition of several cancer targets such as the Abelson proto-oncogene and ABL-related gene, platelet-derived growth factor receptor and a stem cell factor receptor (Habeck 2002).

Chemically, IM is 4-(4-methyl-piperazin-1-yl-methyl)-*N*-[4-methyl-3-(4-pyridin-3-yl-pyrimidin-2-yl-amino)-phenyl]-benzamide methane sulfonate. Extensive literature survey did not reveal any UV-spectrophotometric method for analysis of IM. Few analytical methods are reported in literature for determination of IM in formulations and various biological matrices (Vivekanand et al. 2003; Ivanovic et al. 2004; Szczepek et al. 2007). All methods use sophisticated analytical instruments such as capillary electrophoresis, HPTLC, HPLC-UV and LCMS, making them unsuitable for routine analysis (Velpandian et al. 2004; Guetens et al. 2006; Widmer et al. 2004). Thus, a simple, rapid and cost effective analytical method is required for routine analysis of IM in bulk and pharmaceutical formulations.

The objective of the present study was to develop a sensitive, selective and robust UV-spectroscopic method for determination of IM in bulk drug, pharmaceutical formulations such as tablets, nanoparticles and other quality control samples. The method was validated as per standard validation guidelines (ICH and USP) for analytical methods using suitable statistical tests. Further, the method was successfully employed for determination of solubility of IM at various pH.



Imatinib

2. Investigations, results and discussion

Preliminary investigations of IM in different solvent systems, buffer phases (pH 1–13) and formulation matrix have shown a UV absorbance spectrum with minimum change at 285 nm, which was selected for further optimization (Fig. 1a, b). Finally, the selected solvent system (methanol : 10 mM, pH 7.4 phosphate buffer 50 : 50 v/v) has demonstrated advantages in terms of sample prepara-

Table 1: Statistical data summary for spectroscopic method

Parameters	Spectrophotometric method
Calibration range	2.5–25 μg/mL
Linearity (Regression coefficient)	$R^2 = 0.9995$
Regression equation	Absorbance (AU) = 0.047 × Conc. (μg/mL) + 0.008
Confidence interval of slope[a]	0.0456 to 0.0485 (Std. error = 0.001)
Confidence interval of intercept[a]	−0.0299 to 0.0146 (Std. error = 0.008)
Standard deviation of intercept ($\acute{S}_c$)	5.76×10^{-3}
t-value for intercept[a, b] (tab = 2.57)	0.955 (P-value 0.39)
F-value (tab)[c]	2.88×10^{-4} (1.80)[c]
Standard error of estimate	8.10×10^{-3} (0.300 μg/mL)
Limit of detection	0.57 μg/mL
Limit of quantification	1.71 μg/mL
Absolute recovery	99.18–102.04%
Precision (%RSD)	Repeatability − 2.21% (intra-day) Intermediate precision − 0.80% (inter-day)
System suitability	System precision − 0.85% (n = 10) Molar absorptivity − 2.75×10^3 L/M cm Specific absorptivity − 4.66×10^2 mL/g cm Sandell's coefficient − 2.15×10^{-2} μg/cm^2
Selectivity (resolution)	selective at 285 nm
Robustness	Organic component ± 20% Buffer strength ± 100 mM pH ± 1%

[a] calculated at 0.05 level of significance
[b] calculated using the test of the intercept ($t_{df} = |C - \alpha|/\acute{S}_c$)
[c] calculated using Fisher test with one-way ANOVA (P-value < 0.05)

tion, analyte stability, time and cost of sample processing and wide applicability. The molar and specific absorptivity of IM was found to be 2.75×10^3 L/M cm and 4.66×10^2 mL/g cm in the optimized solvent system while the Sandell's coefficient was found to be 2.15×10^{-2} μg/cm^2 (Table 1). Thus, the optimized wavelength has not only demonstrated merits in terms of better sensitivity and repeatability but also selectivity from formulation matrix with significant pH tolerance.

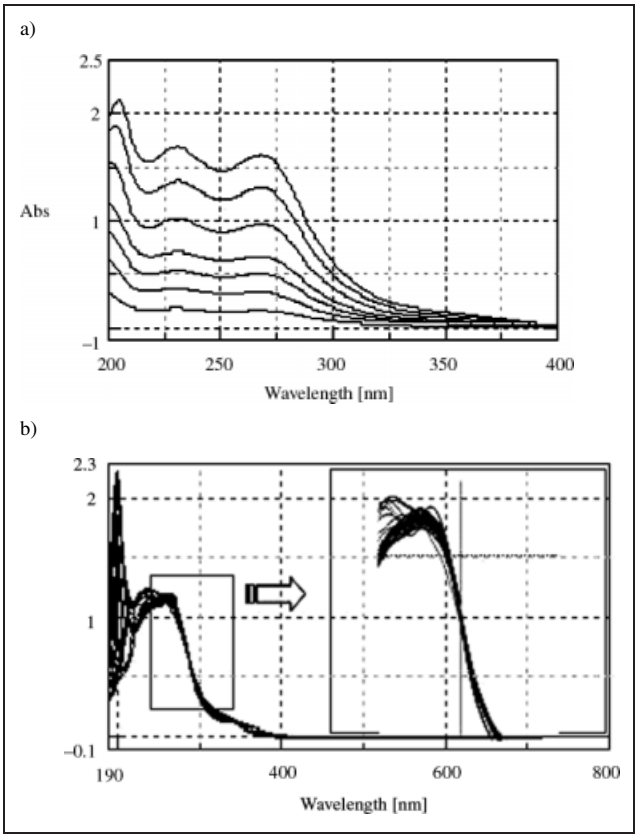


Fig. 1a: UV-Visible absorption spectrum of imatinib mesylate
a) in 50 : 50 v/v methanol: PBS (1–25 μg/mL)
b) in buffer and unbuffered media (pH 1–13)

The absorption spectrum of placebo samples of both the formulations did not show significant interference in determination of IM. Moreover, formulation standards and test samples showed no significant change in absorption spectrum when compared with fresh calibration standards. The method has demonstrated high and consistent recoveries at all concentration levels. Further, the calculated t-values were less than the critical t-value which confirmed that there was no statistically significant difference between the mean absorbance of formulation and calibration standards (Table 1). Thus, it may be suggested that the proposed method demonstrates adequate selectivity for IM in presence of formulation excipients and other impurities.

The least square regression analysis indicated excellent linearity over the range 2.5–25 μg/mL with linear relationship ($r^2 = 0.9995$). The best-fit linear equation obtained was average absorbance (AU) = 0.047 × concentration (μg/mL) + 0.008. Across the analytical range, standard deviation of absorbance was significantly low (± 0.001) and %RSD was below 5 (Table 2). Selected linear model with univariant regression showed minimum %bias indicating goodness of fit which was further supported by low standard error of estimate and mean sum of the squared residuals.

Table 2: Calibration curve of imatinib mesylate by the spectrophotometric method

Concentrations (μg/mL)	Average absorbance[a] ± Standard deviation	%RSD	Predicted concentrations	%Bias
2.5	0.1137 ± 0.004	3.87	2.55	2.01
5	0.2276 ± 0.001	0.19	4.98	0.49
7.5	0.3522 ± 0.006	1.64	7.63	1.74
10	0.4565 ± 0.003	0.55	9.85	1.48
15	0.6889 ± 0.003	0.43	14.80	1.32
20	0.9447 ± 0.003	0.28	20.25	1.27
25	1.1646 ± 0.002	0.21	24.94	0.26

[a] Each determination is average of fifteen replicates

Table 3: Recovery studies by placebo spiking and standard addition technique

Products	Technique	Amount of drug added (% of label claim)[a]	Mean absolute recovery (%)	%RSD	% Bias
Tablets	Placebo spiking[b]	50	100.78	1.57	0.78
		75	99.67	1.64	0.33
		100	99.18	1.43	0.82
		125	100.71	2.00	0.71
		150	99.75	1.02	0.25
	Standard addition[d]	0[f]	99.52	0.66	0.48
		50	99.40	0.58	0.60
		100	100.05	0.42	0.05
Nanoparticles	Placebo spiking[c]	50	101.47	2.52	1.47
		75	101.91	3.04	1.91
		100	101.94	0.99	1.94
		125	100.99	1.75	0.99
		150	101.08	1.62	1.08
	Standard addition[e]	0[f]	100.22	3.37	0.22
		50	102.04	3.10	2.04
		100	101.68	3.10	1.68

[a] Each level was processed independently and analysed in six replicates
[b] Placebo tablet matrix equivalent to unit dose weight
[c] Placebo nanoparticulate preparation equivalent to unit dose
[d] Commercial tablet preparation containing 100 mg of equivalent Imatinib
[e] In-house prepared nanoparticulate preparation containing 10 mg of equivalent Imatinib
[f] recovery results of formulations

The effect of formulation matrix was statistically insignificant as the intercept was not different from zero, which was confirmed by the test of the intercept using t-statistic ($t_{df} = 0.955 \ll t_{table} = 2.57$). Finally, one-way ANOVA was performed on individual absorbance recorded at all concentration levels and the calculated F-value was less than the critical F-value at 5% significance level (Table 1).

In recovery studies, the methods showed consistent and high absolute recoveries at all five concentration levels. The mean absolute recovery values ranged from 99 to 102% for both tablet and nanoparticles. Placebo spiking and standard addition technique indicated that the obtained absolute recoveries were normally distributed around the mean (100%) with uniform and low %RSD (0.42 to 3.10) across five concentration levels, which suggested the suitability of univariant regression model. Thus, it can be summarized that there was no significant interference of excipients and the method was found to be accurate with low %bias (0.05 to 2.04). Recovery study indicated that the method was suitable for determination of IM from tablets and nanoparticles (Table 3).

Precision was determined as repeatability and intermediate precision. Freshly prepared three QC standards ($n = 6$; at each level) showed no significant variation in measured response demonstrating repeatability of the method with %RSD below 2.21. Similarly, inter-day %RSD was significantly low (≤ 0.80) indicating intermediate precision of the method. Low %RSD demonstrated the repeatability and intermediate precision of the method (Table 4).

The LOD and LOQ of the method were found to be 0.57 and 1.71 μg/mL, respectively (Table 1). The method has shown a high magnitude of slope and a low standard error. Upon repeated analysis at quantitation limit, the mean absolute recovery was consistently high with acceptable %bias and %RSD. Thus, the method was found to be

Table 4: Results of repeatability and intermediate precision study

QC Levels	Repeatability (Intra-day)						Intermediate precision (Inter-day)	
	Day (I)		Day (II)		Day (III)			
	Mean[a]	%RSD	Mean[a]	%RSD	Mean[a]	%RSD	Mean	%RSD
LQC	2.53	1.76	2.53	2.21	2.50	1.22	2.52	0.80
MQC	10.04	1.29	9.94	1.31	10.05	1.05	10.01	0.61
HQC	25.28	1.30	25.23	1.11	25.31	1.09	25.28	0.16

[a] Each determination is average of six replicates

Table 5: Robustness studies – mean absolute recovery from quality control standards

Standards	pH		Buffer Strength		Composition	
	(pH 6.5)	(pH 8.4)	(0 mM)	(200 mM)	A	B
LQC	100.4 ± 1.47	100.9 ± 0.91	97.9 ± 2.02	102.3 ± 1.41	102.7 ± 1.62	103.6 ± 1.73
MQC	101.0 ± 0.81	100.9 ± 1.82	98.3 ± 1.06	101.6 ± 1.14	102.6 ± 2.09	103.4 ± 1.25
HQC	101.3 ± 0.69	100.5 ± 1.00	99.7 ± 0.73	101.5 ± 0.81	101.7 ± 0.63	102.1 ± 0.68

Each value is mean of three independent determinations
Composition A is methanol and phosphate buffer (pH 7.4, 100 mM) (30 : 80, v/v) and B is acetonitrile, methanol and phosphate buffer (pH 7.4, 100 mM) (20 : 50 : 30, v/v)

highly sensitive as a small change in drug concentration can be accurately determined by the proposed method.

The statistical analysis indicated that the obtained response remains unaffected by small variations in critical method parameters such as pH, buffer strength and composition of solvent media. In addition, there was no significant change in absorption spectrum of IM in near vicinity of detection wavelength (275–295 nm). The results obtained from statistical analysis of individual treatment showed that the Student's t-values and F-values did not exceed the tabulated values indicating no significant difference between the treated and untreated group, as far as accuracy and precision of the method are concerned. The mean absolute recovery and %RSD for individual factors suggest that the proposed method was robust (Table 5).

The method was found to be suitable in terms of system repeatability as the results were found to be consistent with low variability in absorbance (%RSD < 1.5). Further, the absorption spectrum of drug exhibited no significant change for 48 h at room temperature when compared against freshly prepared standards. The results indicated that the drug was stable in solvent media at ambient temperature and %RSD was found to be less than 2.78.

An absorption spectrum of IM extracted from commercial tablets and in-house prepared nanoparticulate formulations were consistent with the pure drug standard. The mean recoveries were found to be in good agreement with the labeled claim of individual products. The method was found to be accurate and precise with consistently high recoveries of 99.52 (%RSD 0.66) and 100.22% (%RSD 3.37), for tablet and nanoparticles, respectively. Moreover, both the formulations showed low %bias, which indicated that the interference of formulation excipients was insignificant (Table 3). Thus, the method was found to be suitable for determination of IM from both formulations.

The pH metric solubility analysis indicated that the aqueous solubility of IM is highly charge dependent (Fig. 2). In both un-buffered and buffered systems, a trend of decreasing solubility with increasing pH was observed. A sigmoidal relationship existed between solubility and pH of media with relatively constant behavior at acidic and alkaline pH. IM was found to be highly soluble (756 ± 76.15 mg/mL) at extreme acidic conditions (pH 1), whereas at extreme alkaline conditions (pH 12) it was relatively poor (0.04 ± 0.004 mg/mL). One way analysis of variance (ANOVA) indicated that there is no statistically significant difference between the results obtained from UV-spectroscopic method and liquid chromatographic method. Thus, the proposed method was found to be selective and accurate for determination of IM.

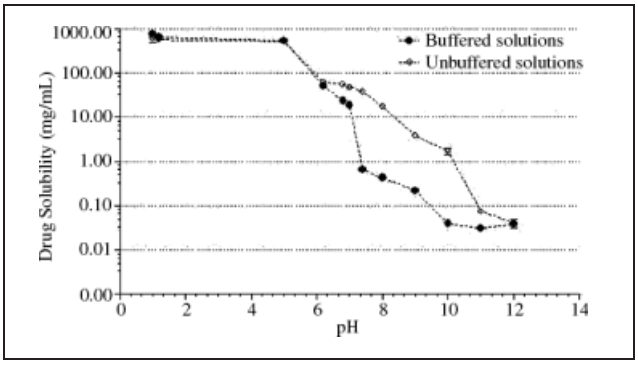


Fig. 2: pH metric solubility profile of imatinib mesylate
---●--- "Buffered solutions"
---○--- "Unbuffered solutions"

3. Experimental

3.1. Materials

Imatinib mesylate (Assay 99.95%) was generously provided by Cipla Ltd., India as a gift sample. Extra pure spectroscopic grade acetonitrile, methanol etc. were purchased from Spectrochem, India. All buffer salts were of analytical grade and purchased from S.D. Fine Chemicals Ltd., India. Ultra pure water was prepared fresh using a Milli-Q® water purification system (Millipore Co., USA) and filtered (0.22 μm) before use. All other chemicals and reagents were of either spectroscopic or highest analytical grade. Poly(lactide-co-glycolic acid) copolymer (PLGA) was provided as a test sample by Purac, USA.

All excipients used for the preparation of placebo tablet blend such as diluent (Avicel PH101 NF), glidant (Aerosil NF), lubricant (magnesium stearate NF), disintegrant (Polyplasdone XL-10 NF), binder (Hypromellose USP), etc. were kind gift from Medreich Pharmaceuticals, India. One commercially available tablet preparation – Imalek 100 (Sun Pharmaceutical Ltd., India) labeled to contain 100 mg equivalent Imatinib was selected from the local Indian market. IM loaded PLGA nanoparticles were prepared in-house using solvent evaporation technique. Similarly, blank PLGA nanoparticles prepared without drug was used as placebo standards.

3.2. Instrument and spectrophotometric conditions

The spectroscopic measurements were carried out using a V-570 double beam UV-VIS spectrophotometer (Jasco, Japan) in 10 mm matched quartz cells. Data acquisition and analysis were performed using Spectramanager® workstation (Jasco, Japan). Absorption spectrums were recorded from 190 to 800 nm at a speed of 400 nm min^{-1} with 0.2 nm data interval, using medium response and 1 nm bandwidth. The quantitative analysis was carried out in photometric mode at a fixed wavelength of 285 nm.

3.3. Methods

3.3.1. Method development

Initially, different solvent systems (pure water, methanol, acetonitrile, 0.1 N HCL, 0.1 N NaOH, phosphate buffer (pH 1–13) and combination of above solvents) were studied to develop a selective and sensitive UV-spectroscopic method for the analysis of IM in formulations. The criteria employed for solvent selection was ease in sample preparation, analyte stability, preparation time, cost of sample processing and comprehensive applicability. Wavelength was selected by assessing critical performance parameters such as sensitivity, reproducibility, selectivity and robustness. Absorbance of IM standard solutions in optimized media at a selected wavelength was determined and the molar absorptivity and Sandell's sensitivity were calculated according to standard formulae. Robustness of the method was studied by changing pH, buffer strength and composition of solvent media. Analysis was performed at ambient temperature (25 °C) after instrument stabilization for at least 15 min.

3.3.2. Preparation of stock and standards

A primary stock solution of 100 μg/mL was prepared by dissolving 10 mg of IM in 100 mL of optimized solvent media consisting of 100 mM phosphate buffer (pH 7.4). Seven calibration standards containing 2.5, 5, 7.5, 10, 15, 20 and 25 μg/mL of IM were prepared by transferring aliquots of stock solution into series of 10 mL standard flasks and volume was made up with solvent media. On each day of validation, three separate series of seven calibration standards were prepared fresh and absorbance values were recorded in photometric mode at 285 nm against blank solvent system.

Formulation standards were prepared by adding known amount of drug in placebo blend of tablets and blank nanoparticulate preparation at five levels – 50, 75, 100, 125 and 150% of the labeled claim. Similarly, placebo standards were prepared in both the matrices without adding drug. Prepared standards were processed and analysed as described.

3.3.3. Sample preparation

An accurately weighed amount of product (tablet and nanoparticles) equivalent to 10 mg of IM was transferred to 100 mL calibrated flask. Only in case of nanoparticles, preparations were digested with 10 mL of acetonitrile by ultra-sonication (15 min, 25 °C). The volume was made up to 100 mL with solvent media and samples were centrifuged (10,000 rpm, 15 min, 20 °C) after mixing for 5 min. Finally, 1 mL of clear supernatant was transferred to 10 mL calibrated flasks and diluted to volume with solvent media and analyzed by the proposed method.

3.3.4. Method validation

The developed spectroscopic method was validated for selectivity, linearity and range, precision, accuracy and sensitivity. The method was also applied for the drug content analysis from commercial tablets and in-house prepared nanoparticle formulations. Additionally, this method was success-

fully employed in pH-solubility profiling of IM in buffered and un-buffered aqueous solutions.

Placebo and drug spiked placebo standards (Formulation standard) of tablets and nanoparticles were used to study selectivity of the method. On three consecutive days, two sets of placebo and formulation standards were prepared in triplicate. On each day of validation, one set of standards was used to study absorption spectrum from 190 to 800 nm, while the other set of standards was used for absorbance measurement at 285 nm for quantitative estimation of IM. The obtained spectra were compared for change in absorption profile with the spectra of fresh calibration standards. The means of absorbance at each concentration level were compared using paired t-test at 95% level of significance.

Calibration standards prepared at seven concentration levels ranging from 2.5 to 25 μg/mL were analyzed for establishing linearity of the method. The least square linear regression analysis was performed on average absorbence values at each concentration level. One-way analysis of variance (ANOVA) was performed on each replicate measurement obtained at seven concentration levels. Analytical range of the method was established by analysis of residuals and test of the intercept was carried out using t-statistics.

Quality control (QC) standards prepared at lower (LQC = 2.5 μg/mL), medium (MQC = 10 μg/mL) and higher (HQC = 25 μg/mL) concentration levels from independent stock solution were used to assess the repeatability (intra-day) and intermediate (inter-day) precision of the method. Six series of three QC standards were prepared freshly and analyzed for assessing repeatability (three times in a day) and intermediate (three consecutive days) precision of the method. Precision of the method was expressed as percent relative standard deviation (%RSD) of assay results.

To assess accuracy of the proposed method two different recovery techniques were carried out for both the formulations (viz. tablets and nanoparticles) separately. In placebo spiking technique, a known amount of pure drug standard was added to placebo blank at five concentration levels viz. 50, 75, 100, 125 and 150% of the labeled claim of the individual formulations. In standard addition technique, a known amount of pure drug standard was added to preanalyzed sample solution at 50 and 100% concentration level of the labeled claim of individual formulation. On three consecutive days, each concentration level was processed in six replicates and the results are expressed as mean absolute recovery, %RSD and %bias.

The sensitivity of the method was assessed using calibration standards. Sensitivity was expressed as limit of detection (LOD) and limit of quantitation (LOQ), which were determined using standard deviation of intercept (σ) and slope (s) of the calibration curve. The LOD and LOQ of the method were calculated using a standard formulae 3.3 σ/s and 10 σ/s, respectively.

Robustness of the method was assessed by making small variation to the internal method parameters. The effect of critical operating parameters such as pH (± 1 units), buffer strength (± 100 mM) and addition of organic solvent – methanol and acetonitrile (+ 20%, v/v) was investigated. At each media condition (pH, buffer strength and organic solvent), one set of QC standards (LQC, MQC and HQC) was prepared in triplicate and the results are expressed as mean absolute recovery, %RSD and %bias. For statistical analysis, one-way ANOVA was carried out to study the effect of each factor.

System precision was evaluated by performing repeatability of the calibration standards with ten replicates. Further, stability of the drug in solvent media was tested by analyzing stock solution and calibration standards in triplicates over 1 month and 48 h duration, respectively.

Twenty tablet samples were finely powdered after weighing and quantity of powder blend equivalent to 10 mg of IM was weighed accurately and processed as described. For nanoparticle samples, a quantity of freeze dried nanoparticles equivalent to 10 mg of IM was weighed accurately and processed as described.

The validated analytical method was employed for pH metric solubility analysis of IM in buffered and unbuffered solutions (1–12 pH). Buffered and unbuffered solutions were prepared as per standard procedure at a constant ionic strength using sodium chloride (USP XXIX, 2005). The extreme acidic and basic solutions were prepared using hydrochloric acid and sodium hydroxide, respectively. Solubility analysis was carried out using a shake-flask method reported elsewhere (Bard et al. 2008). Briefly, an excess amount of the drug was added to 5 mL of solvent media into a glass vial and it was vortex-mixed for 5 min. Mixtures were maintained under constant agitation at 25 ± 0.5 °C using a water bath shaker. After equilibration period of 24 h, determined in a separate study, the samples were subjected to centrifugation (10,000 rpm, 10 min, 25 °C). The clear supernatant was transferred to a fresh glass vial and diluted with solvent media. All sample processing was performed under isothermal conditions to avoid precipitation of drug. Samples were analysed immediately by the proposed method. In order to demonstrate accuracy of the method, all the samples were also analysed by a stability indicating liquid chromatographic method (Bende et al. 2007). All solubility experiments were performed in triplicate and the average solubility of IM in various buffered media was calculated.

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