Research Article

Development and validation of a sensitive RP-HPLC method for quantitative determination of imatinib mesylate in complex dosage forms like drug eluting coronary stents

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ABSTRACT

Aim: The present study is aimed to develop and validate an accurate, precise, and simple reversed-phase–high-performance liquid chromatography (HPLC) method for the estimation of imatinib mesylate (IM) released from the IM-coated stents. **Materials and Methods:** Different chromatographic conditions were tried for IM separation in the placebo (uncoated stents) and validated as per ICH guidelines. The validated HPLC method is applied to measure IM released from IM-coated stents. **Results:** The chromatographic separation was achieved on C18 column (250 mm × 4.6 mm i.e., 5 µm) with a mobile phase consisting of methanol-water-glacial acetic acid (65:35:0.03%, v/v) at a flow rate of 1.0 ml/min. Detection was carried out at 267 nm with photodiode array detector and IM eluted at 5.02 min (retention time) with no interfering peak of excipients used for the preparation of dosage form. The method was linear over the range from 0.3 to 9 µg/mL (R^2 = 0.999). The intraday and interday precision values were <2%. Limit of detection and limit of quantitation were 0.2 µg/mL and 0.5 µg/mL, respectively. **Conclusion:** The method validated according to ICH guidelines and successfully applied for quantitative analysis and release studies of complex pharmaceutical dosage form like IM-coated stent.

KEY WORDS: High-performance liquid chromatography, Imatinib mesylate, Phosphate buffer saline, Polylactic-co-glycolic acid

INTRODUCTION

Imatinib Mesylate (IM) is 4-4[(4-methyl-1- piperazinyl) methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl] amino] phenyl] – benzamide mono methane sulfonate [Figure 1]. IM is one of the signal transduction inhibitors, blocks the tyrosine kinase activities of abl and bcr/abl, and has been utilized to treat patients with chronic myelogenous leukemia.[1] The current research showed that IM is one of the potential platelet-derived growth factor receptor inhibitors, and it may be a potential molecule to inhibit stent-induced smooth muscle cell proliferation in coronary arteries.[2] Implanting coronary drug-eluting stents (DESs) are one of the widely accepted treatments for coronary artery disease patients. Sirolimus, everolimus, zotarolimus, and paclitaxel are commonly used drugs in current generation DES and their loading dose on coronary stents is very less when compared to conventional dosage forms. To measure the loading dose and *in vitro* release kinetics (>30 days) of such low drug-loaded stents are very challenging and need a sensitive analytical method. The reported high-performance liquid chromatography (HPLC) methods estimated IM in pharmaceutical dosage forms (tablets and liquids) and human/rat plasma samples (therapeutic drug monitoring and pharmacokinetics studies).[3-5] There is no reported sensitive HPLC method to measure the IM content in novel and complex pharmaceutical formulations such as DES and their release media. The present research article focused on these aforementioned points. The results of the analysis were according to ICH guidelines.[6]

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Website: jprsolutions.info	ISSN: 0974-6943

MATERIALS AND METHODS

Materials

IM was a gift sample from Hetero labs (Hyderabad, Telangana, India). Methanol (HPLC grade), chloroform (HPLC grade), and glacial acetic acid

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Received on: 08-11-2018; Revised on: 24-12-2018; Accepted on: 19-01-2019

(analytical grade) were purchased from Merck, India. Phosphate buffer saline (PBS) pH 7.4 was purchased from Hi-Media laboratories Pvt., Ltd., Mumbai. Double distilled water for analytical purpose was obtained from Milli-Q R-O system (SG Analytical, India). Poly (DL-lactide-co-glycolide) (polylactic-co-glycolic acid [PLGA], 50:50 with inherent viscosity 0.32–0.48 dL/g) was generously supplied by Purac (Netherlands) in the form of PURASORB PDLG 5004A and Cobalt chromium bare-metal coronary stents were generously supplied by MeKo Laser Material Processing (Germany).

IM-coated Stents and its Release Kinetics

The bare cobalt chromium coronary stents were coated using a formulation containing IM and PLGA by dip-coating method as described in Figure 2. Briefly, 25 mg IM and 50 mg PLGA were dissolved in 50 ml chloroform in a glass beaker. The mandrel loaded stent (Step 3 in Figure 2) is periodically dipped into the beaker and removed and dried under vacuum to remove the residual solvents. The dried IM-coated stents were stored at −20°C until used for further analysis.

Chromatographic Conditions

The HPLC system consisted of a Shimadzu LC-20AT LC pump, Rheodyne injection port (Rheodyne, Cotati, CA, USA) with a 20 µl sample loop, and SPD-M20A photodiode array (PDA) detector (Shimadzu, Kyoto, Japan). Data collection, integration, and calibration were accomplished using 21 CFR part 11 compliant LC solutions chromatography data system. The chromatographic separation of IM was accomplished using 250 mm × 4.6 mm Phenomenex C18 5 µm reverse-phase analytical column. The optimized mobile phase consisted of methanol, water, and glacial acetic acid in the ratios of 60:40:0.03%, v/v. The mobile phase was filtered by passing it through a 0.45 µm Whatman filter paper and the filtrate is degassed using bath sonicator. The PDA detection wavelength was set at 267 nm, the mobile phase was pumped at an isocratic flow of 1 ml/min at room temperature, and analysis was performed after baseline stabilization for at least 30 min.

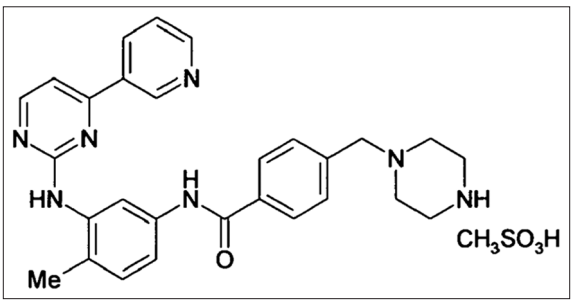

Figure 1: Imatinib mesylate chemical structure

Preparation of Standard Stock Solution

The standard stock solution was prepared in 50-mL volumetric flasks with 50.0 mg of IM weighed exactly and dissolved in methanol. The solution was sonicated for 10 min and adjusted to the volume with methanol. The working standards for IM (0.3–9 µg/mL) were freshly prepared from the standard stock solutions by serial dilution with *in vitro* release rate medium (PBS, pH 7.4) and stored at −20°C.

Preparation of Spiked Recovery Sample

To pick up the possible recovery problems during both the chromatographic procedures and the sample preparation, the spiked recovery samples were prepared by spiking known levels of IM into the product matrix (placebo). The following amounts IM: 10, 50, and 90 µg were spiked into the product matrix and diluted to the volume of 10 mL with the release medium. Three replicate samples of each level along with the respective controls (IM spiked in the release medium only) were prepared. All samples were incubated at 37°C in the release rate bath for 24 h before analyses.

In vitro Release Studies

The *in vitro* release rate profile of eluting stents loaded with IM was studied using laboratory shaking water bath, where each stent is placed in a 2 mL microcentrifugation tube dipped in 1 mL release rate medium (37°C ± 0.5°C) at a rate of 40 rpm for 1, 7, 21, 30, and 45 days. At the end of each time interval, the solutions were transferred into HPLC vials and

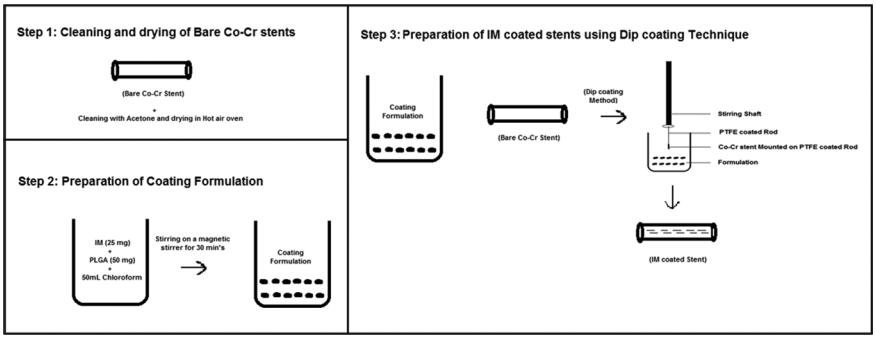

Figure 2: Schematic representation of dip-coating method

injected into HPLC column for the quantitation of IM released. After the last time point, the stents were analyzed for the residual of IM. They were separately transferred into 2 mL vials containing 1 mL methanol, and these vials were sonicated for 30 min. The extracted solutions were transferred into HPLC vials and analyzed for IM.

RESULTS

Selection of HPLC Conditions

The initial stage of the proposed research work consisted of a number of qualitative experiments to determine which column, mobile phase, column temperature, and flow rate should be employed. On the bases of these studies, a Phenomenex (C18, 250 mm × 4.6 mm, 5 µm) reverse-phase analytical column was selected for our application. The selection of the mobile phase was the second most important step in the development of this method. From literature, it is very clear that methanol and water will separate IM from other molecules in most of the matrix, and to sharpen the peaks, we added different concentrations of glacial acetic acid and finally with 0.03% glacial acetic acid, 60% methanol, and 40% water as mobile phase, we get IM sharp peak at 5.02 min. The total analysis time required was only 7 min per sample. The retention time as an index of compound quality is the most important parameter for the length of analyses. The short run time reduces the time consumption for routine series of analyses and, moreover, reduces the solvent consumption.

Validation

The parameters essential to ensure the acceptability of the performance of the analytical method were determined such as the system suitability, specificity, linearity and range, precision, accuracy, solutions stability, and the limit of quantitation (LOQ).

Table 1: IM system suitability

Injection	Rt	Area	*n*
1	5.023	62760	13074
2	5.023	63215	13043
3	5.023	63449	12999
4	5.023	62991	13056
5	5.023	62954	13059
Mean	5.02	63073.8	13046.2
RSD	0	0.42	0.22

Rt: Retention time, *n*: Number of theoretical plates, RSD: Relative standard deviation, IM: Imatinib mesylate

System suitability parameters

The system precision was assessed by analyzing five injections from the same sample (1.0 µg/mL IM). The results of the system suitability testing are given in Table 1. For the definition of the efficiency, various parameters can be used.[7] In our study, the theoretical plate number was used. Apparently, the chosen chromatographic conditions provided a large enough *n* value for the separation [Table 1], indicating that the selected column was reliable and had the ability to produce sharp, narrow peaks achieving a good resolution of band pairs. All of the values meet the ICH requirements for validation (relative standard deviation [RSD]<2).

Specificity

The specificity was evaluated by an injection of a mixture of the working standard solution, fresh and aged *in vitro* release media, and the placebo stent extract. No interference was observed at the same or within ±5% of the retention time of the IM [Figure.3].

Linearity and range

Standard curve was constructed for IM in the range of 0.3–9 µg/ml concentration range by fitting a regression line to the test results (peak area vs. analyte concentration) using the method of least squares. Seven standards were used to define adequately the relationship between the concentration and response. The sum of squares for residuals was equal to zero, indicating that the calibration lines for each analyte were described by a linear relationship. The following equation was derived from the calibration curve: $y = 61978x$, $R^2 = 0.9993$. This was used to calculate the concentrations of IM released in release media and any unknown samples assayed with that analytical run.

Accuracy and precision

The accuracy and precision with which known concentrations of IM in the release rate samples can be

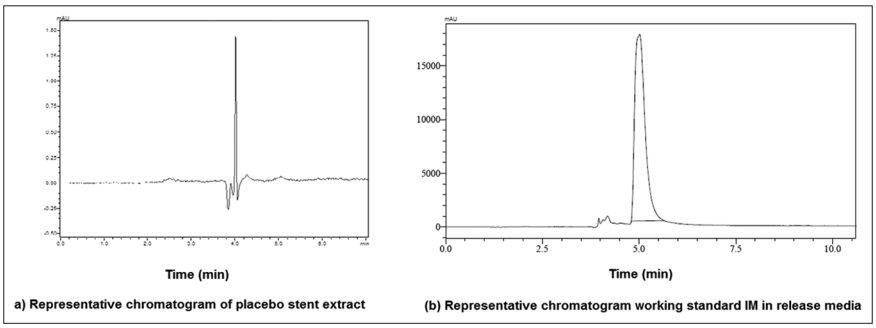

Figure 3: Representative chromatogram of placebo stent extract and working standard imatinib mesylate in release media

determined were evaluated. The recovery assessment was performed by analyses of samples spiked with known amounts IM at three concentrations representing the entire range of expected concentrations in the unknown samples. The mean recovery ranged from 99.3% to 100.2% [Table 2]. Intra-assay precision (repeatability) was assessed using nine determinations from the accuracy study covering the specified range for the procedure (three concentrations/three replicates for IM) and the grand percentage RSD was 0.48% [Table 2]. The interday (intermediate) precision was assessed by running nine spiked samples of IM on three different days, in one laboratory. The samples were prepared freshly at each day. The grand percentage RSD was <2% [Table 3].

Stability studies

The studies were performed to gain information on the stability of standard and sample solutions under defined storage conditions and assure that the solutions are stable enough to allow for delays such as instrument breakdowns. The stability of the standard stock solutions was evaluated over a 28-day period at –20°C; working standard solutions were tested at 2–8°C and room temperature over a 7-day period, and sample solutions were tested at 2–8°C, room temperature, and 37°C over a 3-day period. In addition, the stability of the standard stock solutions was determined after three freeze-thaw cycles. The standard stock solutions were stored at −20°C for 24 h, thawed unassisted for 8 h at room temperature, and refrozen for 24 h under the same conditions. The freeze-thaw cycle was repeated two more times and the samples were analyzed on the third cycle. The stability of IM was tested using six independent sets of sample preparations. The acceptable stability was defined as ≤5% change in the standard or sample response, relative to freshly prepared solutions (T0). The quantitation of analytes was determined using freshly prepared standards. There was no significant loss in IM as shown by percentage difference concentration values obtained during stability tests [Tables 4 and 5].

Limit of detection (LOD) and LOQ

The LOD/LOQ for IM was determined based on signal-to-noise (S/N) ratio approach. Determination of the S/N ratio was performed by comparing measured

Table 2: Accuracy and intraday precision

Spiked amount (µg/mL)	Mean recovery (%)* (%RSD)	Grand mean recovery (%)** (grand %RSD)
1	99.3 (0.45)	99.7 (0.48)
5	100.2 (0.33)	
9	99.6 (0.51)	

*Mean values represent three spiked samples for each concentration. ** Grand mean values represent nine spiked samples (three samples at three concentrations) analyzed on the same day. RSD: Relative standard deviation

Table 3: Interday precision*

Spiked amount (µg/mL)	Mean recovery (%) (%RSD)/day			Grand mean recovery (%) (grand %RSD)
	1	2	3	
1	99.35 (0.92)	101.07 (1.15)	100.31 (1.01)	100.14 (1.36)
5	101.21 (0.88)	100.63 (0.95)	100.85 (0.91)	
9	99.71 (1.01)	100.81 (0.87)	100.23 (1.20)	

*Mean values represent three spiked samples for each concentration. Grand mean values represent 27 spiked samples. Nine spiked samples IM on three different days, in one laboratory. RSD: Relative standard deviation, IM: Imatinib mesylate

Table 4: Stability of IM in standard solution

Standard solution/conditions	% difference from To		
	IM - 1 µg/mL	IM - 5 µg/mL	IM - 9 µg/mL
Long-term stock solution (28 days; –20°C)	1.24	0.97	1.13
Freeze-thaw (three cycles) of stock solution	–0.36	1.05	0.33
Working standard (7 days; 2–8°C)	1.08	–0.76	0.85
Working standard (7 days; room temperature)	0.89	–1.23	–0.93

IM: Imatinib mesylate

Table 5: Stability of IM sample solutions

Sample solution/conditions	% difference from To		
	IM - 1 µg/mL	IM - 5 µg/mL	IM - 9 µg/mL
3 days at 2–8°C	0.13	–0.22	0.17
3 days at room temperature	0.2	0.36	0.31
3 days at 37°C	–0.23	–0.31	–0.34

IM: Imatinib mesylate

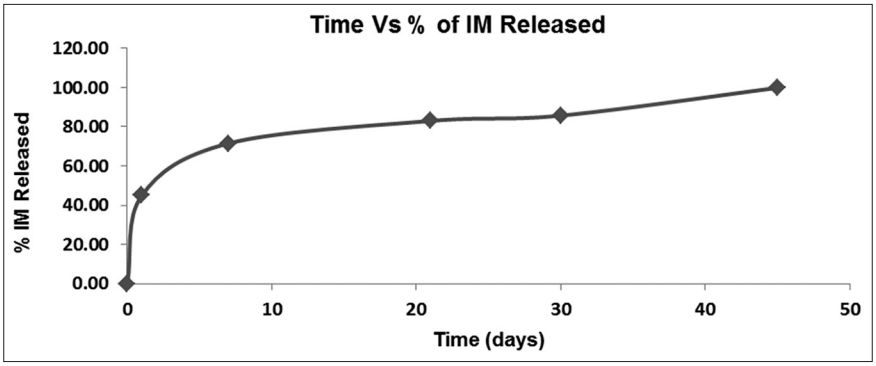

Figure 4: Release kinetics of imatinib mesylate (IM) form IM-coated stents

signals from samples with known low concentrations of IM (0.01, 0.02, 0.03, 0.04, and 0.05µg/mL) with those of blank samples. The lowest concentration that gave the S/N = 3 (i.e., LOD) was 0.02 µg/mL and the concentration that gave S/N = 10 (i.e., LOQ) was 0.05 µg/mL. The LOQ was subsequently validated by the analyses of five independent samples spiked with known amounts (0.05 µg/mL) of IM into the product matrix. The mean recovery was 99.5% and percentage RSD was 1.31%. Therefore, IM LOQ defined at 0.05 µg/mL. Due to the low LOQ, the assay allows the quantitation of IM even at ranges that are more typically addressed by LC–mass spectrometry.

Release Kinetics of IM-coated Stents

After validation of the HPLC method, the method used to measure the quantity of IM released from IM-coated stents and the results are shown in Figure 4.

DISCUSSION

Compared with previously published assays for the determination of IM, the present assay has several advantages. It is the first assay reported for IM for stents application. The total run time of 7.0 min is considerably shorter than the other methods. The present assay allows analyses of IM without prior sample cleanup and pre-concentration, and IM can be separated and quantified directly in the presence of excipient (PLGA) and *in vitro* release medium, which could create significant bias if a spectrometric method. Direct injection eliminates multiple sample pre-treatment and pre-concentration steps involved in the previous assays, which require a large quantity of sample volume to achieve sufficient sensitivity and a significant amount of time by the analyst to complete this task. It avoids IM degradation or loss associated with the use of cartridges and sample concentration and assures more consistent, accurate, and quicker results. Only a small volume (25 µL) of sample solution is needed for the analysis, which is of particular interest when small volumes of the release medium are employed for the evaluation of the *in vitro* release rate of eluting stents loaded with very low drug amount, especially IM. The present assay could provide an LOQ even lower than the previously reported one for the routine therapeutic monitoring of IM.

CONCLUSION

The HPLC method described here is sensitive, selective, linear, and reproducible for the determination of IM in complex pharmaceutical formulation like DES and applicable to measure IM released from IM-coated stents in its *in vitro* release kinetics studies.

REFERENCES

1. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, *et al*. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 1996;2:561-6.
2. Park YJ, Min SI, Jung IM, Lee T, Ha J, Chung JK, *et al*. Different responses of neointimal cells to imatinib mesylate and rapamycin compared with normal vascular smooth muscle cells. Vasc Specialist Int 2014;30:5-10.
3. Widmer N, Béguin A, Rochat B, Buclin T, Kovacsovics T, Duchosal MA, *et al*. Determination of imatinib (Gleevec) in human plasma by solid-phase extraction-liquid chromatography-ultraviolet absorbance detection. J Chromatogr B Analyt Technol Biomed Life Sci 2004;803:285-92.
4. Nageswari A, Reddy KV, Mukkanti K. Stability-indicating UPLC method for determination of imatinib mesylate and their degradation products in active pharmaceutical ingredient and pharmaceutical dosage forms. J Pharm Biomed Anal 2012;66:109-15.
5. Bianchi F, Caffarri E, Cavalli S, Lagrasta C, Musci M, Quaini F, *et al*. Development and validation of an high performance liquid chromatography-tandem mass spectrometry method for the determination of imatinib in rat tissues. J Pharm Biomed Anal 2013;73:103-7.
6. ICH Steering Committee. Guideline on ICH Q2B Validation of Analytical Procedures: Methodology, European Agency for the Evaluation of Medicinal Products. London: International Commission on Harmonisation, No. (CPMP/ICH/281/95); 1996.
7. Nováková L, Solich P. A comparison of performance of various analytical columns in pharmaceutical analysis: Conventional C18 and high throughput C18 zorbax columns. J Chromatogr A 2005;1088:24-31.